Metal Accumulation in Net-Spinning Caddisflies, Hydropsychidae, of the Rouge River, Michigan

by

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Preface

The Rouge River watershed has an area of approximately 467 square miles in southeast Michigan of the United States. The watershed includes parts of Detroit, Michigan and 47 other municipalities home to over 1.3 million people. More than 50% of the area is urbanized while less than 25% is undeveloped. Sampling of the Hydropsychidae was conducted by volunteer groups organized by Friends of the Rouge, a nonprofit dedicated to improving the health of the Rouge River. The volunteers collected the insect larvae by wading the headwaters and collecting the caddisfly larvae and other benthos with a D-frame net.

The Rouge River watershed consists of four sub-watersheds that correspond to the branches of the river: Main, Upper, Middle, and Lower. The land drained by each of the four branches is predominantly used for medium density residences. According to the 2011 U.S. National Land Cover Database, the area of land used for commercial sites is 14% for both the Main and Upper River Branches; the commercial land area for Middle and Lower Branches are 9%, and 7% respectively. Industrial land use for the Main, Upper, Middle, and Lower Branches cover 7%, 4%, 10%, and 9%, respectively, for each sub-watershed. The metals analyzed by this study included: Al, Cr, Mn, Co, Ni, Cu, Zn, As, Se, Sr, Cd, Ba, Pb, Na, Mg, K, Ca, and Fe. Analysis at the river branch level showed that mean larval concentrations of Al, Mn, Co, As, and Ni, significantly varied at the critical p value of 0.05. Arsenic accumulation ranged from 0.45 to 20.4 ppm in samples throughout the watershed. On average, larvae from the Main Branch accumulated the most As (3.1 ppm ± 0.44 SE). The mean As accumulation for the other three branches of the River were between 2.0 and 2.1 ppm.

At the watershed level, average metal accumulation over the four sampling years from 2006-2015, varied significantly (p: 0.05) for Al, Mn, As, Cd, Ba, and Pb. Lead peaked with a mean ± SE of 7.5 ± 2.9 ppm in 2008. This same year also produced the larva with single greatest accumulation of Pb at 127 ppm. Conversely, the lowest average of 3.7 ± 0.71 ppm Pb came from the 2015 sampling. Larvae collected in 2012 had the lowest average cadmium accumulation of 0.15 ppm ± 0.015 SE. In contrast, the mean in 2006 was 0.29 ppm Cd ± 0.043 SE. The largest recorded level of Cd accumulation was 1.2 ppm, from a 2006 specimen. Within the Main River Branch mean levels of Cr, Mn, Cu, As, Cd, Pb, and Ba differed from 2006-2015. The average Cr concentration in 2008 was the greatest at 5.24 ppm ± 0.49 SE in the Main River Branch. In this branch, five of the highest ten concentrations of Cr within the caddisflies were from 2008 collection.

From 2006-2015 the mean values from Hydropsychidae of the Upper River Branch varied with respect to As, Al, Co, and Ba. Average As and Co concentrations reached their maximums in 2015 at 898 ppm ± 142 SE and 2.3 ± 0.35 SE respectively. In the Middle Branch of the Rouge River, a comparison of mean metal concentrations from 2006-2015 showed significant (p: 0.05) differences between at least two years with respect to Mn, As, Se, Sr, Cd, and Ba. Caddisfly larvae collected from the Middle Branch in 2015 had the highest average Ba concentrations of 58.0 ppm ± 7.4 SE. In 2006, the mean Ba accumulation was at a minimum of 31.1 ppm ± 4.3 SE in the Middle Branch. These differences in Hydropsychidae metal concentration highlight the changing health of river branches from 2006-2015.

Using a biological indicator such as Hydropsychidae can also be useful to compare watersheds associated with different land uses. Such a comparison can elucidate the impact of human activities including agriculture, industry, and urbanization. Understanding the role of human activities in elevating the concentrations of metals in living organisms is important in preserving the health of a watershed. Here, I compared the concentrations of metal in Hydropsychidae collected from the Rouge River to larvae of the same family from watersheds in Poland, Spain, and Brazil. The regions in Brazil and Poland are heavily urbanized, while the watershed in Spain has varied use including industry and agriculture. More specifically, sites used for comparison included locations associated with: mining, agriculture, industry, and chemical dumping. Relative to the metal accumulation of Hydropsychidae in these other watersheds, the mean concentrations of Cu, Fe, Zn, Cd, Cr, Pb, As, and Ni from the Rouge River insects were relatively low. Unlike the other metals measured, the average Mn concentration in larvae from the Rouge was substantially higher (2.8 times) than an area directly downstream of a known chemical dumping site in the Ebro River of Flix, Spain. Manganese concentrations ranged from 80 to 8,412 ppm in insects from the Rouge River. The caddisfly larvae captured from the Main River Branch had an average concentration of 1096 ± 123 SE ppm Mn, whereas the Upper, Middle, and Lower River Branches were home to larvae with greater averages between 1450 and 1550 ppm Mn.

Examining the long term spatial and temporal extent of metal contamination in caddisfly larvae has far reaching implications. This utility extends to other freshwater ecosystems to help better manage the health of watersheds. Net-spinning caddisfly can be captured and analyzed to help monitor the toxic effects of heavy metals in a watershed. As watersheds become more developed, point and non-point sources of pollution can become more prevalent.

Hydropsychidae can serve as a ubiquitous indicators of metal accumulation that inform stakeholders about the health of streams, sub-watersheds and watersheds.

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Abstract

Biological indicators are useful proxies for assessing water quality. By analyzing historic biological samples collected over many years, stakeholders can evaluate the health of a watershed and how it has changed over time. Measuring metal accumulation over a decade can reveal positive, negative, and geographical trends in a watershed. During the late 1990s and early 2000s, chemical analysis of the bed sediment, surface water, soil, and shallow groundwater indicated the Rouge River Watershed had elevated levels of toxic metal. Directly sampling soil, sediment, and water may not always accurately reflect the uptake of metals by living organisms. Therefore, a direct biological indicator is needed to more definitively examine the impact of toxic metals. Toxic metal contamination is an issue because at heightened concentrations many metals inhibit growth, development, motility, and neural function in animals; some are also known carcinogens.

Here, I measured the metal concentrations in aquatic larvae of net-spinning caddisflies (Hydropsychidae) collected from the Rouge River watershed in 2006, 2008, 2012, and 2015. This type of caddisfly larvae can tolerate a wide range of metals, which makes them useful as a biological indicator because they will persist even in degraded streams, and can also be collected and analyzed years later. For example, the accumulation of Pb ranged from 0.5 – 127 ppm (mg/kg). Similarly, a wide range of concentrations were detected for Cr (0.2 – 16.4 ppm), Mn (80 – 8413 ppm), As (0.4 – 20.4 ppm), and Ba (6.0 – 329 ppm). Using 182 specimens of this

biological indicator, I identified spatial and temporal patterns of metal accumulation across the Rouge River watershed.

Hydropsychidae are suitable monitors for metal pollution, and can be used to complement other sampling methods to evaluate the extent of contamination in a watershed. The metal contents of sediment and surface water are limited in their ability to express the biological impact of metal contamination as the exposure pathways, uptake, and elimination of metals is heterogeneous across different taxa. The tissue analysis of these larvae can more accurately express the deleterious impact of toxic metals on freshwater organisms.

Chapter 1: Introduction

Heavy metals have been a persistent and significant pollutant in communities with a history of industrialization; the Rouge River Watershed of Southeastern Michigan is no exception. Previous studies revealed elevated levels of trace metals throughout the watershed in the surface water of the Rouge (Murray et al., 1997), the shallow groundwater (Murray et al., 2006), bed sediment (Murray, 1996; Murray et al., 1997; 1999), and soils (Murray et al., 2004). These sinks and sources of trace metals all interface with the Rouge River. Metals may enter the river through runoff, atmospheric deposition, and the influx of groundwater. With such pervasive contamination, the organisms living in the watershed are likely to be exposed and affected. To assess the extent of metal contamination total metal concentrations in soils and water are oftentimes used.

However, a growing body of evidence indicates that total concentrations of metals in sediment and surface waters may not predict bioavailability, the portion of metals that directly impact organisms (Vink, 2002). The uptake of the metals by organisms is not homogenous (Maiz et al., 2000). Two filter feeding benthic invertebrates, *Ephoron virgo* and *Hydropsyche exocellata*, had significantly different (α: 0.05) metal accumulation with respect to Cd, Ni, Cr, Pb, Ti, and Zn (Cid, 2010). Furthermore, the body metal concentrations of *E. Virgo* significantly differed (α: 0.05) for certain metal with development along instars (Hg, Cd, Cr, Pb, Ti, and Mn) and also between sexes (Cd, Cu, and Mn) (Cid, 2010). Sampling and the analysis of tissues should complement other measures of contamination from soil, sediments, air and water. The metal accumulation within taxa should be considered in determining the environmental impact of the many human activities that can concentrate pollutants in water, and benthic sediments.

Metals can become further concentrated in living organisms. In freshwater ecosystems, many metals (Al, Mn, Ni, Zn, Cr, Co, Cu, Fe) have been shown to bioaccumulate (ratio of body/sediment concentration >1) in a variety of benthic macroinvertebrates (Chiba et al., 2011). Aquatic macroinvertebrates also show increasing concentrations of Pb up the food chain (Goodyear and McNeill, 1999). Greater concentrations of metals have been linked to lower biodiversity in freshwater macroinvertebrate communities (Sola et al., 2004; Sola et al., 2006). In fact, metals can impact an entire ecosystem. Metals have been shown to reduce biodiversity in macroinvertebrate communities (Sola et al., 2004 and Sola et al., 2006), and this negative impact likely extends through the aquatic and riparian ecosystems. Heavy metal contamination in streams reduces fungal colonization and the rate of leaf litter decomposition (Ehrman et al., 2008). Consequently, a bottom-up reduction at trophic levels could affect the whole aquatic community. Many benthic invertebrates are at lower trophic levels, metal contamination may have a cascading impact, and so using tissue samples from net-spinning caddisflies would be an important measure of stream and riparian health.

Caddisfly larvae can serve as a bio-indicator of the bottom-up ecosystem impact of metal pollution. Metal accumulation at lower levels of the food chain may provide an indirect assessment of the trophic system. For example, emergent aquatic insects, like Hydropsychidae, serve as a food source for riparian predators as well. High concentrations of methyl mercury have been found in riparian spiders which are a food source for some songbirds (Speir et al., 2014). Methyl mercury concentrations increased with trophic level from herbivorous aquatic insects up through freshwater fish species (Speir et al., 2014). Many fish species feed on benthic invertebrates such as Hydropsychidae. The Agency for Toxic Substances and Disease Registry (ATSDR) has reported increased levels of metals such as cadmium and lead in many freshwater fish including walleye and pike. The ATSDR, a United States federal public health agency, works to protect communities from environmental hazards, such as the consumption of fish with dangerous levels of toxic metals. Chronic exposure over many years to low levels of metals and pollutants leads to severely depressed reproduction function in fish and may even eliminate local populations altogether (Oost, 2003). Elevating levels of metals in Hydropsychidae could be indicative of metal accumulation in predators such as fish and spiders. Thus, using net-spinning caddisflies as a bio-indicator is not limited to judging the health of the benthic community. The larvae can be used a bio-indicator that can identify larger stream and riparian communities that may be at risk to toxic metal accumulation.

Here we use specimens from 2006 - 2015, to assess the concentration of heavy metals in net-spinning caddisflies. Samples spanning a nine year period provides an assessment of the dynamic health of the river ecosystem. Many studies provide a snapshot of metal pollution, but none give a historical perspective over longer periods of time. Historical samples were taken from more than 50 sites (Figure 5) from the Upper, Lower, Middle and Main Branches of the Rouge River. Using these sites, I conducted a spatial analysis in addition to a temporal investigation of metal accumulation in net-spinning caddisflies.

Background on the Rouge River Watershed

The Rouge River and its tributaries are approximately 790 miles in total length. The Rouge River Watershed is an urban area of 467 square miles. This area can be further broken up into sub watersheds, the Main, Upper, Middle, and Lower Branch. Each of the sub-watersheds consist of tributaries and land that feed a larger branch of the river. The four large branches of the river meet in the southeastern corner of the watershed to form the stem of the Rouge River. The stem is 5.5 miles long and empties into the Detroit River. The watershed has an average population density (Figure 1) of 2800 people per square mile. About 82% of the land area is developed. Deciduous forest covers 6.2 % of the watershed, and open waters make up 4%. Wetlands, cultivated cropland, and pastures each cover about 2% of the area. The watershed receives, on average, 30 inches of precipitation annually. The EPA has permitted 1055 National Pollutant Discharge Elimination System (NPDES) permits for point source pollution.

In 2004, Murray et al. tested soils throughout the watershed for 13 metals. Most had high levels associated with industrialized and urbanized areas (Murray et al., 2004). This trend was evident in surface and subsurface soils (Murray et al., 2004). Soils contaminated with metals have pathways to the river. One of those paths is through runoff from precipitation. During wet weather events, the Rouge River is flashy with highly variable discharge because runoff can reach the river quickly (Wayne County, 2011). The flashiness of the river is in part due to impervious surfaces associated with the majority of the watershed's developed land. Storm runoff can sweep surface soils sorbed with metals into the river.

Heavily urbanized and industrialized areas on the southeastern side of watershed have more impervious surfaces. United States Geological Survey (USGS) stream gauges indicate that high flow variability still impacts the quality of the Rouge (Price, 2013). Stable streamflow is ideal for aquatic life in streams. During heavy rainfall and storms the stream flow can increase rapidly especially in urbanized areas such as the Rouge River watershed. A rapid increase in flow can suspend sediments and mobilize metals in the river. One metric that indicates high flow variability is the frequency of peak flow events. In the case of the Rouge River, peak flow is when stream gauges measure a flow of greater than 700 cubic feet per second. From 1990 to

2013, the peak flow events in the Main and Upper Branches have generally decreased (Wayne County, 2013). During the same time period, peak flows in the Lower and Middle Branches have increased at some sites and remained constant at others (Wayne County, 2013). These peak flow events provide an enhanced pathway for sediments laden with metals to reach the Rouge River.

Murray et al. (1999) discovered higher levels of metals bound to fine sediment compared to coarse. Typically, the lower reaches of a river are dominated by fine sediment. Indeed, samples of bed sediment revealed an increasing portion of Fe, Cr, Ni, Pb and Zn at downstream sites (Murray et al., 1999). The type of soil may also influence the exposure risk to metals in streams. Some metals are more or less mobile in a particular type of soil. Increased mobility in the soil enhances the path of metals to a river. Relatively higher levels of mobility for Hg, Cr, and As have been observed in clay soils (Murray et al., 2006). Cadmium and Cr mobility are elevated in sandy soils (Murray et al., 2006). This mobility may contribute to metal exposure at the sites within these associated depositions. Sands and clays that originated from glacial lakes dominate the surface soils in the southeastern two thirds of the watershed (Rouge River Watershed Management Plan, 2012). Deposits from glacial moraines makeup most of the northwestern portion of the watershed (Rouge River Watershed Management Plan, 2012). For both groundwater and sediment, which may interface with the river, metal pollution generally increases from less urbanized to more urbanized (Murray et al., 2004; Murray et al., 2006).

Industrial point sources have also polluted the Rouge with metals. In 1994, The Michigan Environmental Response Act identified 100 sites of environmental contamination within the Rouge River watershed (Bean et al., 1994). Of those 100 sites, 28 have confirmed contamination of heavy metals (Figure 3) (Bean et al., 1994). In the Main Branch of the river, fourteen sites were classified with metals as a major pollutant (Bean et al., 1994; Beam and Braunscheidel 1998).

The Upper, Lower, and Middle Branches included 0, 5, and 9 metal polluted sites respectively (Bean et al., 1994; Beam and Braunscheidel, 1998). Zinc and lead contamination was the most widespread and severe in the sediments of the watershed (Beam and Braunscheidel, 1998). Generally, the sediments of the Lower Branch had the highest metal content with decreasing concentrations moving toward the northern portion of the watershed (Beam and Braunscheidel, 1998). The entire last 5.5 mile section of the main stem of the Rouge was labelled a site of environmental contamination due to elevated levels of Pb, Cr, Ba, Cu, Zn and many organic pollutants (Beam and Braunscheidel, 1998).

Benthic Invertebrate Biodiversity of the Rouge River

In addition to historical point sources, examining the biodiversity of benthic macroinvertebrates may also serve as a predictor for metal accumulation. Higher biodiversity is associated with lower levels of metal pollution (Sola et al., 2004; Sola et al., 2006). However, increasing levels of flashiness, storm water discharge, sewage infiltration, and industrial discharges may lower the biodiversity of these communities (Selzer, 2008). In the fall of 2013, regions near the origin, or headwaters, of the Middle Branch had the greatest biodiversity (Figure 4) (Wayne County, 2013). Many of the sites with relatively high biodiversity are also located closer to the headwaters (Figure 4) (Wayne County, 2013). This trend is not consistent throughout the watershed. Some areas of poor or fair biodiversity are adjacent to sites with more species. The three poorest sites of biodiversity are all along or near one of the four larger branches (Figure 4) (Wayne County, 2013). The four larger branches are fed by confluence of many smaller tributaries and run through the four sub-watersheds: Main, Upper, Middle and Lower.

Although biodiversity may be used as a predictor of metal contamination in part, many other factors (dissolved oxygen levels, water temperature, turbidity, and organic pollutants) influence the community structure of benthic macroinvertebrates. The Rouge River has a legacy of stress and contamination. In 1992, the River was classified as an environmental Area of Concern (AOC) by the International Joint Commission (Selzer, 2008). The beneficial use impairments included the restriction of fish consumption, loss of habitat, and the degradation of wildlife, including benthos (Selzer, 2008). In 1992, a federally funded initiative deemed the Rouge National Wet Weather Demonstration Project began efforts to restore the River (Alliance of Rouge Communities, 1992). The project continued until 2014 and included resolutions to reduce or remove the following: combined sewer overflows, surface runoff, siltation, flashiness, erosion, and contaminant loading (Wayne County 1992-2014).

Since 1999, the diversity of benthos and wildlife has consistently risen (Alliance of Rouge Communities, 2012). Pollutant sensitive benthic macroinvertebrates have returned to some tributaries (Wayne County, 2014). Remediation efforts have likely reduced the loading of heavy metals into the river. For example, Wayne County began collections of electronic and household hazardous waste (Wayne County, 2011). The county has also targeted commercial and industrial facilities suspected of illicitly discharging deleterious materials (Wayne County, 2011).

Characteristics of Net-Spinning Caddisflies, Hydropsychidae

Aquatic invertebrates, such as caddisflies, have many qualities that make them suitable bio-monitors. Caddisflies are in contact with metals sorbed to suspended or benthic sediments. These filter feeders are found in most streams throughout North America (Berner and Wiggins, 1977). Hydropsychidae larvae live in tubular retreats attached to rocks or submerged debris. Unless disturbed, the larvae are sedentary which is a useful characteristic when monitoring the contamination at a site. At the end of the retreat these caddisflies construct a silk net to capture and consume particles from flowing water. The life cycle of Hydropsychidae varies by geographic location. In most temperate locations, this family of insects may have 1 or 2 generations a year (Zuellig, 2004). Larvae go through 5 instars before the pupal stage takes place within a cocoon until the emergence of a winged adult. Caddisfly larvae can tolerate a wide range of physical and chemical conditions. The insects are known to inhabit streams with significantly different ($p< 0 .05$) levels of dissolved oxygen, water conductivity, pH and discharge rate (Tszydel et al., 2015). Net-spinning caddisfly larvae are abundant within the Rouge River and can be collected over a large spatial and temporal scale. Net-spinning larvae are less sensitive than other types of caddisflies and they have been used to as an indicator of metal contamination in multiple studies (Sola et al., 2004; Sola and Prat, 2006; Tszydel et al., 2015; Vuori and Kukkonen, 1996). Researchers have also used Hydropsychidae to measure the uptake and elimination rates of heavy metals in solution (Evans et al., 2002; Evans et al., 2006).

Three genera of Hydropsychidae larvae inhabit the Rouge River. The family is distinguished by three sclerotized (darkened and hardened) dorsal plates (Figure 6) found on the thorax of the larvae. *Ceratopsyche*, *Hydropsyche*, and *Cheumatopsyche* are the three genera collected from the Rouge River. The *Ceratopsyche* and *Hydropsyche* genera include larva with two large sclerids (darkened and hardened plates) on the ventral thorax beneath the most superior pair of legs (Figure 7). *Hydropsyche* larvae have a solid dark brown head, and in some cases light rings around the eyes. In contrast, *Ceratopsyche* larva have a variable number of light spots on top of the head (Figure 8). The spots may differ in size, location, shape, and number. Insects belonging to the third genera, *Cheumatopsyche,* have a notch on the anterior edge of the clypeus (Figure 9) in the larva.

Factors Influencing Spatial Distribution of Metals

This study used larvae from the Hydropsychidae family, which consists of net spinning caddisflies. These filter feeders may ingest metals through particles they encounter in flowing water and captured in their silk nets. The amount of metal contamination in the suspended sediment, bed sediment, and water all likely contribute to accumulation in caddisflies. Nevertheless, even in controlled laboratory studies, metal uptake is highly variable (Evan et al., 2002, Evans et al., 2006). Metal concentration in caddisflies varied considerably throughout the watershed.

Elevated concentrations of heavy metals can be hazardous to all living organisms. Heavy metals occur naturally, their distribution and concentration can be severely altered by human activities. Evaluating the metal contamination of streams can be complex. Many factors can contribute to the varied concentrations encountered. For example, differing climates, land use, and geology can all influence the extent of contamination (Sakan et al., 2015). Thus, direct comparisons between sites to assess the level of contamination can be challenging. Many heavy metals are toxic to a variety of living organisms. Such contamination must be treated as a hazard.

Industrial processes are responsible for the loading of metals into aquatic ecosystems. For example, in 59 sediment samples, Cd, Cr, and Ni were enriched by a factor between 2-3 times within sediment from the Yangtze River (Weiguo et al., 2009). This contamination is associated with approximately 1.25 x106 tons of untreated industrial and domestic waste discharged into the river daily (Shanghai Water Authority, 2007).

Land use can play a significant part in elevating the levels of metals within a watershed. Industrial activities, mining, and agriculture have all been associated with high levels of metal pollution. Lead enrichment can reach approximately 5 times the naturally occurring Pb in such areas (Sakan et al., 2015). Additionally, enrichment can peak 14 and 36 times the background concentration for Zn and Cu respectively (Sakan et al., 2015). Agriculture is a known polluter of manganese. Many fertilizers and fungicides contribute to high levels of Mn. River basins with high agricultural use yielded sediments with 4 fold enrichment of Mn (Sakan et al., 2015). Heavy metals settle out of the water to reside in sediment. However, disturbances in the environment such as a change in flow or discharge may mobilize the metals (Sakan et al., 2015). Biota can facilitate the transfer of metals through uptake and elimination. For example, filter feeders and other benthic organisms may assist in redistribution.

Sediment distribution and composition within rivers can influence local concentrations of metals and in turn their bioavailability. Consequently, Hydropsychidae inhabiting different types of bed sediment may be more or less prone to toxic metal accumulation. Multiple physical and chemical features govern the heavy metal content in sediments. The effect size of such characteristics vary. Empirical evidence has shown the composition of clay has one of the largest effects. In decreasing order of effect size, other parameters that influence the metal content of sediments include: organic matter fraction, carbonate fraction, and silt fraction (Howari and Banat, 2001). Organic matter has considerable surface area for sorption along with a high capacity to chelate the metals (Gupta and Subramanian, 1998). Carbonate fractions showed a positive correlation with metal concentrations (Forstner, 1981). This could be due to the metals precipitating with calcium carbonate. Howari and Banat (2001) reported the Jordan River mud fraction contained levels of Pb, Cd and Zn at 6.6, 0.63, and 14 ppm respectively. The Pb, Cd, and Zn concentrations of the clay fraction were 8.1, 0.55, and 20.3 ppm. With exception to Cd, the average metal concentrations in clay were higher. The finer grain size of clay provides more surface area for metal adsorption. In the Yarmouk River of Jordan, average Pb concentrations in, clay and mud were reported as 8.4 and 6.8 ppm respectively. Average Cd levels were 0.67 ppm in clay and 0.6 ppm in mud. For Zn, concentrations were 26.44 ppm in clay, 20.5 ppm in mud. Benthic invertebrates may have a higher risk of metal exposure in fine grain sediments with higher organic and carbonate fractions. This study does not attempt to link the composition of bed sediment to metal accumulation, but different types of bed sediment could have an impact on geographic trends in Hydropsychidae metal accumulation.

Metals with higher mobility have an enhanced pathway to reach freshwater streams and benthic communities. Zinc, Cr, Cu, Ni, Pb show low mobility, while researchers observed a high mobility of Cd (Gao and Chen, 2012). Gao and Chen collected forty-two sediment samples from the Bohai Bay and 8 rivers flowing into the bay. Within the marine environment, metal concentrations were generally not significantly different. Sampling locations within the rivers showed significant trends in the upstream and downstream directions. For example, in the Dou River of China, Cr, Cu, Ni and Pb levels increased downstream. Conversely, the neighboring Yongingxin River contained sediments with lower concentrations of all six metals as sampling moved toward the bay. These maximum concentrations were all observed in the fluvial sediment. Anthropogenic sources could be responsible for the spatial distribution of metals in fluvial sediment.

Much of the metal content of sediments may be unavailable to the biota. The portion of the metals that are bound within the mineral matrix are unavailable because of an association with oxides, carbonates and organics (Howari and Banat, 2001). The available or exchangeable metals have a path to the local organisms. The variation in the availability of different metals makes predicting the biological uptake and impact difficult. Examining accumulation in tissue samples from local organisms may serve as a more suitable indicator of toxic metal pollution than measuring metal concentration in sediments.

Chapter 2: Metal Toxicity

Foreign substances act as toxins when they bind to macromolecules and hinder cellular membrane function, metabolic activity, or genetic expression. Many heavy metals inhibit protein and enzyme functions by binding to sulfhydryl groups within the biological molecules (Kakkar and Farhat, 2005). Such damage at the molecular level oftentimes leads to developmental delays or disease. Generally, an organism can eliminate toxins by expelling them directly or converting them to a more water soluble form before excretion. However, when uptake exceeds the rate of metabolic expulsion, bioaccumulation occurs. The widespread use of metals in industry, coupled with their persistence, increases the exposure risk to biota. For example, nickel (Ni) can enter the environment as a by-product of fossil fuel combustion. The World Health Organization (WHO) has reported elevated levels of Ni near refineries, battery plants, sewage outfalls, and coal ash disposal sites. Even if current precautions limit the paths of metals to the environment, their longevity makes legacy pollution a hazard for years.

Metal toxicity studies have been ongoing since the early 1980s (Cheng and Wang, 1985). Preliminary research focused on determining the acute and chronic toxicities that would be lethal to 50 percent of the population (LC50) or have an effect on 50 percent of the population (EC50) (Wang, 2012). Both LC50 and EC50 are related to the exposure concentration and duration. Elevated levels of metals can cause serious damage to the environment and human health. Aquatic animals can accumulate dissolved metals or consume metals through their diet.

Predators and deposit feeders have demonstrated that diet is the most significant source of accumulation (Wallace et al., 2000). For example, cadmium (Cd) accumulation increased with feeding in grass shrimp (*Palaemonetes pugio*) (Wallace et al., 2000). Bivalves transported to contaminated sites also showed high levels of Cd along with impaired growth and elevated mortality rates (Perceval et al., 2006). Some tissue concentrations have been an accurate predictor of toxicity. In water fleas, *Daphnia magna*, toxicity tests revealed lethal doses of Zinc (Zn) are around 400 ppm (mg/kg) dry weight (Shaw et al., 2006). Toxicity can vary across species. Water flea species, *Cladocerans*, show different levels of tolerance to metal exposure (Shaw et al., 2006). A growing body of knowledge of toxicity specific to each species assists in analyzing contamination risks and effects. Nevertheless, relative accumulation helps guide management efforts within a watershed such as the Rouge. Monitoring for a host of known toxic substances is an imperative part of managing environmental risks in an increasingly industrialized world.

Many metals are confirmed or likely carcinogens. For example, Cd, Ni arsenic (As), chromium (Cr), and lead (Pb) are known human carcinogens. Nickel can inhibit enzymes associated with Zn, calcium (Ca), iron (Fe), magnesium (Mg), and manganese (Mn) (Kasprzak, 1987). In part, this enzyme inhibition is linked to the metal's carcinogenicity. Nickel exposure can lead to genetic damage through the disruption of DNA replication and strand repair (Morales et al., 2016). Neurological damage or cancer caused by some metals can manifest progressively and may cause irreversible damage. For example, lead poisoning causes long-lasting neurological damage. Elevated levels of Pb lead to reduced cognition and a suppression in the sensory functions of many species. Lead impairs many vital signaling and transport pathways that are normally governed by Ca ions (Castro-Gonzalez and Mendez-Armenta, 2008). Metal exposure has inhibited flight behavior, specifically swimming speed, in invertebrates preyed upon by juvenile salmon (McIntyre et al., 2012). Metals pose a wide-ranging and substantial risk to biota. Thus, the identification of contaminated areas is critical in limiting this ecological hazard.

Toxic Effects on Aquatic Ecosystems

The markers for acute or prolonged toxicity can change across species, developmental stage, and be challenging to identify, observe and quantify. Bio-monitors can be used to model and predict the impact of pollutants across many taxa, but they are also key parts of the ecosystem themselves. Invertebrates such as snails and caddisflies are suitable aquatic bio-monitors in part because they represent approximately 90% of aquatic species (Habib et al., 2016). Specific heavy metals and their associated salts are fatal to snails (Habib et al., 2016). Indeed, Barium (Ba), Ni, Zn, and Cu are all known to cause death or stress in a host of aquatic animals (Habib et al., 2016).

The adverse effects of Ni have been documented by the United States Environmental Protection Agency (USEPA) in higher plants, protozoans, insects, crustaceans, fish, amphibians, mollusks, birds and mammals (USEPA, 1975). Nickel lethality ranges from 11-113 ppm for sensitive aquatic species, such as daphnids and some freshwater fish larvae (Schubauer-Berigan et al., 1993). More tolerant taxa, such as freshwater snails, can survive at Ni concentrations upwards of 200 ppm (Jenkins, 1980). Nickel is not only toxic to animals, but plants as well. The USEPA has found that elevated levels of nickel are known to cause a reduction in growth and photosynthetic rate among algae species. Nickel poses a risk to many organisms in freshwater ecosystems.

As with Ni, other metals such as Ba are hazards to animals in streams. Water fleas (*D. magna*) exposed to Ba over 48 hours and 21 days had a 50% mortality rate at 14.5 and 13.5 ppm respectively (Biesinger and Christensen, 1972). In the same study, Ba concentrations of 5.8 ppm induced a 16% decrease in reproductive rates. Moreover, motility ceased for 50% of daphnids at barium sulfate concentrations of 52 and 32 ppm over a 24 and 48 hours respectively. The toxic effects of Ba are not limited to water fleas. High Ba concentrations have been implicated in developmental delays in mussels (Spangenberg and Cherr, 1996). Lethal Ba concentrations for 50% of crayfish samples ranged from 39 to 61 ppm over a 30 day exposure period (Boutet and Chaisemartin, 1973). Exposure to Ba is a threat to animals which should be monitored in rivers.

Bivalves are also a relatively common bio-monitor, and many metals have proven toxic to these organisms (Liu et al., 2011). In species of mussels and clams, As, Hg, Cu, Cd, and Zn have been linked to impaired gas exchange, nutrient uptake, digestion and nerve function (Liu et al., 2011). Increasing Mn levels were associated with reduced gill function in the bivalve, *Crassostrea virginica* (Martin et al., 2008). This reduced function stemmed from Mn disrupting neurotransmitters within the gill (Martin et al., 2008). Manganese is naturally occurring but can be concentrated by human activities. Manganese is required to produce multiple enzymes that are vital for proper neurological and organ functions. However, excessive quantities of Mn inhibit neural activity.

Manganese toxicity in freshwater organisms is likely due to Mn^{2+} ions competing for active sites normally occupied by Ca^{2+} and H+ (Habib et al., 2016). Manganese can also inhibit oxidative enzymes essential to synaptic transmission (Habib et al., 2016). The damaging effects of reduced nerve function are likely compounded by the impairment of locomotion and feeding induced by metal exposure. For example, snails *(Biomphalaria alexandrina*) took 3 times longer to attach to a surface after a 96 hour acute exposure to 0.2 mg Cd/L (Habib et al., 2016). Over the same 96 hour period, both Cd and Mn reduced the distance the snails travelled by 50% and 10% respectively. Snails exposed to Mn at 0.2 mg/L for 96 hours stopped feeding altogether, and the same concentration of Cd reduced feeding by ¾ compared to the control. Snails subjected to a lower dose (0.01 mg/L) of both Cd and Mn over 24 days experienced comparable changes in feeding behavior, locomotion and attachment.

Adverse effects are not limited to the chronic exposure of Cd and Mn. Excessive levels of Zn can inhibit chitinase activity in *D. magna* (Poynton et al. 2007). *Daphnia magna* is a small aquatic crustacean. Chitinase enzymes facilitate the reshaping of the exoskeleton during growth and development. Specifically, the molecule plays a vital role in molting. Chitin is a key component the exoskeleton in arthropods. Many arthropods including *D. magna* must molt in order to reproduce. These crustaceans exhibited a dose sensitive response; the enzyme's activity decreased from 100 ppm until a lethal dose of 1,000 ppm (Poynton et al., 2007). Both chronic and acute doses impaired chitinase activity. Thus, Zn contamination poses a developmental and reproductive hazard to many species.

Additionally, laboratory tests revealed that Cd, Zn, and Cu impaired the production of the digestive enzyme amylase (Poynton et al., 2007). This reduction in amylase may be linked to inhibited feeding behaviors caused by ingestion of metals in other species. Copper also seemed to depress the immune system. Specifically, this metal decreased the expression of molecules responsible for identifying an infectious agent and mobilizing an immune response (Poynton et al., 2007). Therefore, crustaceans exposed to copper would be more susceptible to pathogens. Indeed, mollusks and crustaceans have incurred higher rates of infection after coming in contact with elevated levels of Cu (Parry and Pipe, 2007; Yeh et al., 2004).

Aquatic biota is harmed by the anthropogenic sources of metals such as, Cu, Zn, and Cd. However, some metals, such as Strontium (Sr), have no established water quality standards to protect wildlife. Acute toxicity has been reported to occur over a wide range (75 – 15,000 ppm) by a relatively small number (4) of studies (McPherson et al., 2014).

Cobalt (Co) toxicity also is not well understood. Dissolved acute and chronic thresholds (30-day average) of 110 and 4 ppm Co, respectively, can limit the deleterious effects on ecosystems (Nagpal, 2004). This metal is not common in natural aquatic environments. Cobalt concentrations can be locally elevated by human activities such as mining, agriculture, steel production, and the industrial discharges associated with dyes and batteries (Diamond et al., 1992). Generally, uncontaminated freshwaters have dissolved Co concentration less than 5 ppm (Nagpal, 2004). Higher concentrations (11 – 50 ppm) are usually nearby mining or agricultural activity. Elevated concentrations have been reported as a hazard to some aquatic plants and animals (Nagpal, 2004). Chronic (30-day) toxicity tests indicated the LC50 of 790 ppm dry weight in a species of crayfish (Boutet and Chaisemartin, 1973). Mayflies showed delayed growth at dry body weight concentrations of 32 ppm (Sodergren, 1976).

While many metals pose a direct hazard to aquatic invertebrates, bioaccumulation extends the threat to vertebrates as well. Selenium (Se) can be transferred up the food web by benthic invertebrates who ingest the metal from sediments and detritus (Alaimo et al., 1994). In North Carolina, Se discharged from ash ponds of a coal power plant were largely responsible for the disappearance of 19 out of 20 native fish species (Hamilton, 2004). Another well documented case of the destructive bioaccumulation of Se occurred in Kesterson National Wildlife Refuge in the San Joaquin Valley, California. Selenium ridden leachate from agricultural waste infiltrated the reservoir, causing widespread mortality in multiple fish and bird species (Ohlendorf, 1987). Subsequent studies revealed elevated levels of Se at every trophic level, along with deformations and reproductive depressions in fish and bird populations. Developmental deformities were linked to dietary consumption of Se in birds that lead to egg concentrations of just 5 ppm dry-weight (Ohlendorf et al., 2011). Midge larvae and other benthic populations have flourished at Se concentrations between 76 and 180 ppm and thus are generally not at risk for Se toxicity (Schuler et al., 1990). In contrast, fish and bird populations begin to decline at dietary Se concentrations of 3 and 11 ppm (DeBruyn and Chapman, 2007).

Correlation to Community Diversity

Heavy metal concentrations are negatively correlated with the taxa richness of benthic stream invertebrates (Liess et al., 2017). Australian streams had a correlation between high levels of metals and a reduction in species richness and invertebrate abundance (Liess et al., 2017). Compared to metal contaminated sites with only an average of 9 species, unpolluted locations had 22 distinct species (Liess et al., 2017). Furthermore, the abundance of individuals also declined with an average of 1302 individuals collected at reference sites compared to only 151 from polluted sites (Liess et al., 2017). Laboratory studies have identified the acute toxic effects of metals on aquatic invertebrates. Metal exposure produces a specific assemblage of invertebrates in the laboratory as more sensitive species die off (Von der Ohe and Liess, 2004). These controlled studies do not predict the sensitivity of invertebrates to chronic low dose exposure that may occur in the environment (Von der Ohe and Liess, 2004).

Many freshwater pollutants also depress taxa in terms of abundance, richness, and biodiversity. A specific biological indicator for metal pollution would be valuable in monitoring and identifying threats to aquatic ecosystems. Many community measures are not correlated with heavy metal contamination. An increased ratio of predatory invertebrates has been positively linked with elevated levels of metal. Mountain streams infiltrated by Cu mine waste had a severely depressed population of invertebrates that scrape and graze (Qu et al., 2010). Invertebrates that feed on detritus or plant matter accumulated more metal contaminants than predators (Qu et al., 2010). Indeed, researchers have discovered that many of the most sensitive aquatic invertebrates are not predatory. In North America, two of the most sensitive aquatic species to metal exposure are mayflies. Both of these species ingest plant matter and detritus through scraping and grazing (Clements, 1994). Therefore, toxic metals may disrupt predatory invertebrate populations less, and yield a characteristic ratio based on feeding strategy.

An invertebrate study in Australia exemplifies the impact of metal contamination on benthic communities. Metal contaminated sites had a disproportionately lower abundance of benthic invertebrates that scrape and graze for food. Predatory invertebrates also had a reduction species richness at the contaminated sites. Out of 35 communities of invertebrates from this study, eleven sites were upstream of known point sources, specifically tin and copper mines (Liess et al., 2017). The remainder of the sampling locations were downstream of the mines. Invertebrate predators were more depressed at polluted sites by a difference of 3 species (9.6 to 6.6) and 306 individuals (457 to 141). Copper and Zn were the most elevated, with concentrations in stream water ranging from 2.5 - 9.0 ppm and 18.9 - 194 ppm respectively. The USEPA sets maximum acceptable concentrations at 2.3 ppb as a standard for both Cu and Zn. The assemblage of invertebrates at polluted sites had higher proportions of predators. Predator ratios were 0.32 at locations with low Zn and Cu concentrations, and 0.73 at the upper ends of the concentrations recorded. A Pearson's test indicated a strong linear correlation, $r = 0.78$, between metal exposure and predator ratios (Liess et al., 2017). The populations of invertebrate herbivores and detritivores were reduced even further at the Cu and Zn polluted locations.

Calculating the ratio of predatory invertebrates is a more accessible monitoring option than spectrometry to determine metal concentrations from biota, water, or sediment. Analyzing metals concentrations through spectrometry requires lab instruments that may be inaccessible to non-profits, and river advocacy groups. Sampling and identification can be done by volunteers with more readily available equipment, such as waders, nets, and sampling containers. Calculating the ratio of benthic predators could be a valuable strategy to locate areas contaminated with toxic metals.

Carcinogenicity

The ability of some metals (As, Cr, Cd, and Ni) to cause cancer is rooted largely in their propensity to induce genetic mutation. Many metals cause the formation of reactive species inside of cells. These reactive species, such as free radicals OH, O^{2-}, and $ONOO^{-}$, can damage DNA (Waris and Haseeb, 2006). The free radical OH is especially hazardous because of its ability to react with nitrogenous bases and the sugars in the backbone of DNA. Such reactions can lead to breaks in the double helix (Dizdaroglu, 2012).

Some metals promote cell health at trace levels. Iron is an essential component of proteins involved in cell respiration and gas transporting cofactors such as heme. Excessive levels of Fe can produce mutagenic hydroxyl free radicals (Torti and Torti, 2013). Elevated levels of Fe can cause single strand breaks in DNA (Torti and Torti, 2013). Cancers generally contain higher concentrations of Fe than tissues growing at a normal healthy rate (Torti and Torti, 2013).

Iron is not alone in its ability to maintain cell health at appropriate levels, but promote the formation of cancer at excessive concentrations. Acceptable amounts of Cu aid in the function of multiple enzymes and proteins, but elevated concentrations of Cu can be deleterious. Copper cycles between two oxidation states, Cu^{+} and Cu^{2+}. Both states are highly reactive and mutagenic. Increased levels of Cu are associated with a high rate of cytosine to thymine substitutions (Phatak and Muller, 2015).

Some metal compounds such as arsenic trioxide induce high levels of a tumor suppressor protein called p53 (Filippova and Duersken-Hughes, 2003). Tumor suppressor proteins inhibit cell division when DNA damage poses a risk to cause uncontrolled cancerous growth. The p53 protein repairs damaged DNA or initiates apoptosis if genetic damage reaches a certain threshold. Metal exposure can induce the expression of p53 and destroy the protein at the same time. Zinc plays a critical role in activating the p53 protein. Zinc is essential to the proper folding and function of many essential proteins (Phatak and Muller, 2015). At high concentrations, copper can block the binding of Zn and render p53 inactive (Phatak and Muller, 2015). The inhibition of the cell's tumor defense in tandem with the mutagenic qualities of many metals magnifies their carcinogenicity (Tkeshelashvili et al., 1991).

Toxicity of Cadmium

Some metals pose a greater risk than others due to their toxicity and prevalence. Cadmium, arsenic, and lead are especially hazardous because of their noxious effects on living organisms, and their potential to become concentrated by human activities. Cadmium is well established as an environmental contaminant that is hazardous to many different animals. The WHO set the maximum allowable Cd concentration for potable water at 3 ppb. However, freshwater organisms have demonstrated adverse symptoms from concentrations as low as 1 ppb in the water (Merian and Thomas, 1991). This particular metal has a relatively high solubility in water and bioaccumulates in aquatic organisms. Ionic cadmium (Cd^{2+}) commonly forms compounds such as: cadmium oxide (CdO_2), cadmium chloride ($CdCl_2$) and cadmium sulfate ($CdSO_4$) (Hartwig et al., 2002). In 2003, the Agency for Toxic Substances estimated that between 4,000 and 13,000 tons of Cd were put into the environment by humans. At the molecular level, Cd is known to generate free radicals (Hartwig et al., 2002). More specifically, these free radicals can oxidize nucleic acids and damage molecules that repair DNA. Both Cd and Pb along with some of their associated organometal complexes suppress the immune system (Vos et al., 1989). The toxic effects of Cd vary between species. Generally though, high concentration can lead to death in animals by disrupting calcium homeostasis (Mebane, 2010). The precise control and transport of calcium is integral in neurotransmission and vital enzyme functions (Mebane, 2010.

Metal toxicity is not limited to animals. Plants show adverse effects from contact as well. For example, elevated levels of Cd reduce the function of certain photosynthetic pigments (Garcia-Sevillano et al., 2015). As a result, the plant's rate of growth is stifled. Tomato plants treated with high concentrations of Cd (0.1 molar) grew significantly less than plants exposed to a lower concentration (0.02 molar) (Hediji et al., 2010). Plants and animals can eliminate a certain quantity of metals. Higher quantities of the chelating amino acid asparagine were measured in plants treated with Cd (Hediji et al., 2010). This chelating agent probably eliminated a sufficient portion of the Cd to protect growth in the tomato plants treated with a lower dose of Cd (0.02 molar).

Toxicity of Lead

Lead poisoning has severe neurological consequences. Over the last three centuries, concentration of Pb have increased by more than 1000 times in some areas of the environment as a result of human actions (ATSDR, 2005). The most significant increase took place between

1950 and 2000 (ATSDR, 2005). Lead has an atomic weight of 207.2 and typically occurs in three oxidation states: Pb, Pb (II) and Pb (IV). Elevated levels of Pb cause cognitive damage and a reduction in the sensory functions in many species. At the cellular level, Pb can mimic Ca and inhibit enzymes by bonding to sulfhydryl groups (Castro-Gonzalez and Mendez-Armenta, 2008). Lead disrupts many signaling and transport processes that are normally mediated by Ca (Castro-Gonzalez and Mendez-Armenta, 2008). Moreover, Pb inhibits numerous enzymes involved in the production of heme (Colombo et al., 2004). This cofactor is an essential component in biological molecules responsible for supplying cellular energy and degrading waste products (Paoli, 2002).

Toxicity of Arsenic

Arsenic is a naturally occurring element widely found in rocks and soil. In the environment, As can be found in many organic and inorganic forms. The metal can occur in the elemental form, as 3+ arsenite, or as 5+ arsenate (Casarett et al., 2013). In the organic form, As is combined with hydrogen and carbon. The organic forms are more likely to accumulate in tissues. The biological half-life of inorganic As is about 10 hours, while organic As has a half-life of about 30 hours (Casarett et al., 2013). In contrast, inorganic As is usually found in ground and surface water (Casarett et al., 2013). A wide range of processes can lead to increased As levels in the environment. Some of the largest contributors include mine drainage, pesticide application, wood products, industrial waste, and naturally occurring geothermal discharges (Kakkar and Farhat, 2005).

Acute and chronic As exposure can manifest with the dysfunction of the nervous system, circulatory system, and digestive tract (Graeme and Pollack, 1998). One of the main mechanisms of toxicity for As is its inhibition of enzymes critical for cellular respiration

(Winship, 1984). Arsenic binds to sulfhydryl groups to block active sites of enzymes in the mitochondria including catalysts necessary for the progression of the Krebs cycle (Winship, 1984). Organic As is also a known human carcinogen, and its carcinogenicity likely extends to other animals. Most toxological studies in mammals have been done with rodents. Arsenic damages cells and tissues in numerous ways. In general, this metal disrupts vital metabolic pathways, some of which include amino acid synthesis, energy production and hormone homeostasis (Garcia-Sevillano et al., 2015). Specifically, As inhibits enzymes within the Krebs and slows the production of methionine (Garcia-Sevillano et al., 2015). Additionally, As hampers the degradation of phospholipid membranes and metabolism of choline. These damaging effects at the cellular level led to tissue necrosis in mice and voles (Garcia-Sevillano et al., 2015).

Metal Accumulation

The bioaccumulation of heavy metals poses a serious risk for the biota in an ecosystem. Heavy metals are common pollutants in aquatic ecosystems such as the Rouge River. Higher concentrations can be found in organisms compared to abiotic components of the environment such as particulates, sediments, groundwater, and surface water. Metals easily adsorb to sediments and do not readily degrade. Thus in an aquatic environment, metals pose a direct risk to the benthic organisms. Furthermore, exposure can travel up the food chain by continued feeding and accumulation.

The ingestion of particle bound metals is one path of exposure for aquatic insect larvae (Cid et al., 2010). All aquatic invertebrates will accumulate metals from solution, or to a larger extent, through their diet (Wang, 2002). Laboratory studies have demonstrated that food laden with metals can be a greater contributor to metal accumulation than dissolved sources. For example, amphipods (*Orchestia gammarellus*) accumulated Zn in direct proportion to the concentration of metal in their food (Weeks and Rainbow, 1993). Over a 21 day period, the amphipods accumulation increased as researchers fed the invertebrates seaweed that had been soaked in progressively higher concentrations of Zn.

Water and sediment only hold a portion of the metals available for uptake. Thus, determining the levels of metals in tissue represents a critical component of environmental hazard analysis. Researchers found that high concentrations of metals in suspended particles positively correlate to metal accumulation in caddisfly larvae (Cid et al., 2010). Caddisflies may also ingest metals dissolved in water (Evans et al., 2006) or from food sources (Speir et al., 2014). In addition to caddisflies, a variety of aquatic invertebrates with different feeding strategies accumulate metals dissolved in water and sorbed to sediments (Goodyear et al., 1999). Many fish feed on aquatic insects or their larvae, such as Hydropsychidae. Metals bound to sediments can accumulate in filter feeders like Hydropsychidae. Fish that feed on the contaminated insects subsequently bioaccumulate metals with continued ingestion. The accumulation of Cd and Cu in fish can cause lipid peroxidation, or the oxidation of polyunsaturated fatty acids (Romeo et al., 2000). Lipid peroxidation is associated with a series of damaging chemical reactions (Romeo et al., 2000).

Bioaccumulation is complicated, and dependent on multiple factors. The rate of uptake, elimination, growth, and many other aspects influence the buildup of metals in an organism (Rainbow and Luoma, 2011). Wang et al., (1996) proposed the equation to model the steady state concentrations (C_{ss}) of metals of aquatic organisms. Overall the equation adds together the net intake of metals from solution and food, while at the same time compensating for the growth (g) of the organism (Wang et al., 1996).

The predictive accuracy of the model is limited in some situations when inputs for the model may not be available. When such parameters are available the model should cross-reference actual tissue concentrations. Environmental conditions and the biological diversity of aquatic insects lead to variation in metal accumulation that may not be accounted for in a mathematical model. Accurately predicting bioaccumulation is very difficult because of differences in toxin elimination and uptake across different species and even organs (Oost et al., 2003). Consequently, tissue samples are required to evaluate the buildup of noxious substances.

As metal enters the body of an invertebrate, it will first be available to the cells along the entry pathway and subsequently to other tissues as the metal is transported by the hemolymph. Some trace metals are essential and will be incorporated to organic molecules that help maintain cellular health. For example, carbonic anhydrase contains Zn, and this enzyme plays a role in transporting carbon dioxide and regulating pH in tissues and bodily fluids (Rainbow, 2007). Copper is also an integral component of hemocyanin, a protein that transports oxygen in many invertebrates. Excessive concentrations of metal can be harmful, if not lethal. Decapods, specifically prawns (*Palaemon elegans*), have been observed to progressively accumulate Zn with an increase in dissolved metal concentration (Rainbow and White, 1989). Higher rates of uptake than excretion ultimately led to mortality.

Metals that have no biological function, or accumulate to excessive levels, can be eliminated by detoxifying molecules. Detoxification is largely mediated by a group of molecules called metallothioneins. These molecules regulate the levels of essential metals, such as Cu, Pb, Co, Ni and Zn, in addition to removing non-essential and toxic metals, like Cd (Roesijadi, 1992). In fact, gene expression of metallothioneins is directly linked to the concentration of metals within a cell (Thiele, 1992). This family of proteins removes metals from ligands and free ions and stores them as insoluble granules or sinks in invertebrate tissue (Thiele, 1992). Researchers have classified three types of these granules. Type A granules are made of repeating layers of calcium and magnesium phosphates, and have been determined to contain elevated levels of Mg and Zn (Marigomez et al., 2002). Zinc along with Cu can be stored in granules containing sulfur (type B) that form in a variety of shapes (Marigomez et al., 2002). Lastly, crystalline granules (type C) are predominantly composed of Fe (Marigomez et al., 2002).

Unlike the detoxification through insoluble granules, specialized proteins can remove metals in the soluble phase. Metallothioneins are cytosolic proteins that can prevent metals from interfering with metabolic processes, and contain sulfur rich cysteine amino acids that capture metals (Amiard et al., 2006). Metal rich metallothioneins may be processed by the lysosome to form the previously mentioned sulfur rich granules (type B) (Nassiri et al., 2000). Researchers reported increased levels of type B granules in amphipods inhabiting Cu contaminated waters (Nassiri et al., 2000). In addition to detoxification, excretion can reduce the net levels of metals and help mitigate accumulation. Unfortunately, some invertebrates do not excrete metals; thus, they are more prone to metal toxicity (Rainbow, 2007). The potential to accumulate metals can vary substantially across aquatic invertebrate taxa. Phytoplankton have varying levels of sensitivity to metals, in part, because each species partitions the metals within the cells differently (Wang and Wang, 2008). For example, many decapods can increase excretion as more metals, such as Zn, are ingested (Rainbow and White, 1989). Consequently, decapods will only begin to accumulate metals if their potential for excretion is surpassed by ingestion. On the other hand, barnacles do not have an ability to excrete some metals, like Zn and Cu (Rainbow and White, 1989). Without excretion, the detoxification mechanisms are more easily overwhelmed, and barnacles can accumulate some of the highest levels of metals.

When detoxification and excretion mechanisms are exceeded by the uptake of metals, the toxic effects are magnified. To assess the extent, uptake, and impact of the metal contamination, many researchers use aquatic insects as bio-monitors (Chiba et al., 2011; Cid et al., 2010; Hare and Campbell, 1992; Sola et al., 2004). Multiple studies (Cid et al., 2010; Sola et al., 2004) discovered higher concentration of metals in aquatic macroinvertebrates downstream of a known point source of metal pollution. For example, compared to unpolluted control sites, caddisfly larvae had levels of metals 3-35 times greater (Sola et al., 2004). Laboratory studies corroborated the uptake of metals (Cd, Pb, Zn, and Cu) by the larvae (Evans et al., 2002; Evans et al., 2006).

Chapter 3: Research Methods

Friends of the Rouge is a nonprofit that has routinely collected benthic macroinvertebrates at over 50 sites within the river over the past fifteen years (Figure 5). This organization works to restore and monitor the river. Many of their programs educate the community to promote stewardship of the watershed. One monitoring program, they run biannually, solicits volunteers to collect benthic invertebrates in order to survey the diversity from various locations. These benthics have been archived digitally and preserved physically in 95% ethanol. A group of 182 Hydropsychids collected along all four branches (Upper, Middle, Lower and Main) of the Rouge were analyzed for heavy metal concentrations. Typically, Friends of the Rouge samples in both the spring and fall. Specimens are larger in the spring. Samples were taken from the spring collections as more mature insects can be identified and massed more accurately. To examine the potential of a morphological marker for heavy metal accumulation, the anal papillae were photographed with a light microscope. Previous studies proclaim these structures may become deformed at a threshold level of metal accumulation, which occurs in the form of reduced and darkened protrusions (Vuori et al., 1996). After examining the structure of the papillae the specimens were processed to measure their concentrations of heavy metals in terms of dry body weight.

Samples were analyzed for metal accumulation through the following methods:

1. The Hydropsychidae larvae were vigorously rinsed in ultrapure deionized water to remove metals adsorbed to the exterior surface of the exoskeleton

2. Individual specimens were dried at 60 º C for 24 hours before massing each sample.
3. To assist in digestion, the insects were placed in 2 mL of trace grade nitric acid, and underwent a 35 minute heating cycle in a microwave reactor. The acid and microwave digestion liberated metals bound in the organic samples.
    a. For each digestion cycle, an acid blank accompanied the caddisfly samples as a control measure.
    b. The samples and acid blanks underwent the digestion process in 5 mL glass vials capped with a polytetrafluoroethylene seal and ceramic screw top.
4. A syringe filter removed residual inorganic particles that were not removed by rinsing.
5. Following the digestion, an inductively coupled plasma mass spectrometer (ICPMS) was used to determine the concentrations of each heavy metal in mg/kg dry weight (ppm).
    a. The samples were diluted by a factor of eleven with ultrapure deionized water to allow for ICPMS analysis

Digestion and analysis of the caddisfly larvae allowed spatial analysis based on collection year and site. A further spatial analysis was conducted considering land use. For each the site, the upstream land use was calculated using Long Term Hydrologic Impact Analysis (L-THIA). The L-THIA is a model that predicts the effects of changes in land use on runoff, recharge and nonpoint source pollution (Engel, 2001). Here, it was used to acquire the current upstream land use for each of the Hydropsychidae collection sites (Figure 5). The land uses were placed into four categories: (1) industrial or commercial, (2) residential, (3) agriculture, or (4) undeveloped. Undeveloped land cover included: wetlands, forests, pasture, grasslands, and surface water. These four categories were used because they represented the predominant land cover in the

Rouge River Watershed. The land cover was expressed as a percentage of the upstream drainage area. I looked for correlation between the land use and metal accumulation in Hydropsychidae.

I also investigated the correlation between Hydropsychidae metal accumulation and the Pb, Cu and Cd content of benthic river sediment. The sediment was collected and analyzed at many of the same caddisfly collection sites; sediment data was taken from Murray et al., 1997. Common sites were identified by superimposing two maps showing the sampling sites for the sediment and larvae. Sites were considered common if they were within 0.15 miles up or downstream from one another.

Lastly, I calculated the correlation among metal accumulation and benthic invertebrate biodiversity and richness. The richness is based on the number of different taxonomic orders present. Friends of the Rouge records scores for the biodiversity based on the number of pollutant sensitive, somewhat-sensitive, and tolerant benthic invertebrates; this score is termed the stream quality score (SQI). The SQI scores from the same Hydropsychidae sampling locations and years were provided by Friends of the Rouge for the correlation analysis. Higher scores indicate the presence of more biodiversity and pollutant sensitive taxa. The stream quality score is weighted, and assigns higher values to pollutant sensitive taxa. Some of the sensitive taxa include Hellgrammites (Megaloptera), Mayfly nymphs (Ephemoroptera), Stonefly nymphs (Plecoptera), and Water Penny Beetles (Coleoptera). Some tolerant taxa are aquatic worms (Oligochaeta), Leeches (Hirudinea), Midge larvae (Diptera), and Pouch Snails (Gastropoda). The index sums the weighted score of each taxa to quantify the quality of the stream as excellent, good, fair, or poor.

Quality Control Reference Samples

In order to limit contamination from the manufacturing process, before use caps and vessels were soaked in 20% Alconox detergent for two days. Next, caps and vessels were placed in a one day bath of 5% trace grade nitric. This mitigated metal residues left behind from manufacturing processes. Finally, the caps and vessels were rinsed three times with ultrapure deionized water.

Reference samples from the National Institute of Standards and Technology (NIST) were analyzed using the same procedures as the caddisfly larvae. The NIST standard reference material was used to evaluate the analytical methods in determining the trace concentrations of metals in the Hydropsychidae tissue. Analysis of freeze-dried mussel tissue from NIST standard reference material 2976 revealed the average recovery values were lower than the certified concentrations, except for Se and Cu (Table 1). The certified NIST values were based on 8 samples ranging in mass from 0.0019 to 0.0206 g.

As a control, the acid blanks that went through digestion and dilution were included with the caddisfly samples in the ICPMS. A total of nine acid blanks were analyzed. Contamination was limited to less than one ppm in most cases (Tables 1 and 2). However, the acid blanks had average Zn, Fe, and Al concentrations of 1.01, 1.1, and 3.11 ppm respectively. Relatively less toxic metals such as Ca, K, and Na were also present at higher concentrations (Table 2). The acid blanks show an acceptable level of contamination. Acid blanks had a low concentration, 1.2 ppm or less, of toxic heavy metals. The higher levels of Na, Al, K, and Ca may be from the distilled water used for dilution. The low levels of contamination paired with the conservative metal capture rates of the ICPMS are indicative of adequate methods to analyze the tissue content of invertebrates, including Hydropsychidae.

Statistical Analysis

The statistical tests were preceded by a logarithmic transformation ($\log10(x + 1)$) of the data. This transformation aided in normalizing the data and reducing the variance. A subsequent one-way analysis of variance (ANOVA) tested for significant differences (α: 0.05) between means at different spatial and temporal scales. A Student's T-test compared the mean metal concentrations between larvae with and without protruded anal papillae.

A Pearson's coefficient (r) describes the strengths of associations between metal accumulation and land use. Similarly, this coefficient was used to calculate the correlation between Hydropsychidae metal accumulation and historic metal content (Pb, Cu, and Cd) of benthic river sediment. Lastly, the r values were used to examine the link between metal accumulation and benthic community measures of biodiversity and richness. The strength of correlation coefficients in this paper are described as follows: $0.1 < | r | < 0.3$ weak correlation, $0.3 < | r | < 0.5$ moderate correlation, $| r | > 0.5$ strong correlation.

Chapter 4: Results

Spatial Distribution of Metal Accumulation in the Rouge River Watershed

This analysis of Hydropsychidae metal accumulation is based on the branch level of the watershed. The mean dry body weight concentrations of the insects were compared by their location within the four river branches of the sub-watershed. The four sub-watersheds drain into the Main, Upper, Middle and Lower branches of the Rouge River (Figure 5). The insects are grouped by the branch (Main, Upper, Middle or Lower) from which they were collected (Figure 5). Concentrations are expressed in ppm (mg/kg) dry weight, unless otherwise noted. The number of specimens from the Main, Upper, Middle and Lower Branches are 56, 30, 73, and 23, respectively. A one-way ANOVA compared the means across the branches to determine significant differences.

The 182 samples ranged in aluminum concentration from 68 – 2,029 ppm. On average the Lower Branch had the highest Al body weight concentrations of 797 ppm $\pm$ 94 standard error (SE). A larva from the Upper Branch had the greatest Al accumulation of 2,029 ppm at the field location of Bell1 (Figure 11). Insects from the Main, Upper, and Middle Branches of the River had respective average Al concentrations (ppm $\pm$ SE) of 425 $\pm$ 41, 627 $\pm$ 77, and 547 $\pm$ 73, respectively. Certain field locations had larvae with relatively elevated levels of Al. Fellows Creek (Field ID: Fel4) within the Lower Branch was home to an insect with 1,901 ppm Al (Figure 11). Johnson Creek (Field ID: John8) and Walled Lake Drainage (Field ID: Wall1) are part of the Middle Branch and were home to larvae with 1,839 (John8) and 1,766 ppm Al (Wall1) (Figure 11).

The caddisfly larvae captured from the Main Branch had the lowest average concentration of 1,096 ± 123 SE ppm manganese. The Upper, Middle and Lower Branches were home to larvae with averages between 1,450 and 1,550 ppm Mn. Hydropsychidae inhabiting the Middle Branch accumulated some of the highest levels of Mn at 8,412 ppm (Field ID: Ton1) and 6,299 ppm (Field ID: Ton1) (Figure 12). An insect from Minnow Pond (Field ID: Min3) had a dry body weight concentration of 8,261 ppm Mn (Figure 12). Overall, Mn concentrations ranged from 80 to 8,412 ppm.

On average, cobalt accumulated the most in insects from the Lower Branch. Larvae had mean concentrations of 2.6 ppm ± 0.55 SE in the Lower Branch and averages between 1.47 and 1.58 ppm in the other three branches of the watershed. In total, Co accumulation ranged from 0.37 to 1.6 ppm. Insects from the Lower Branch (Field ID: Low5) had 2 of the 3 highest levels of Co accumulation (11.6 and 9.6 ppm).

Mean selenium concentrations were highest in insects collected from the Main Branch. The mean concentration (ppm ± SE) of insects from the Main Branch was 1.8 ± 0.08 compared to 1.4 ± 0.016, 1.6 ± 0.25, and 1.45 ± 0.10 in the Upper, Middle and Lower Branches respectively. The average body weight concentration varied significantly (p: 0.009) between the Main and Upper River Branches. However, the largest single accumulation of Se was 3.7 ppm from an insect collected at field location of Bell2 from the Upper Branch. This insect accumulated at least 5 ppm more Se than any other specimen. Many larvae with the lowest concentrations of Se came from two locations in the Upper Branch: Up1 and Up2. Selenium accumulation was lowest in an insect at 0.24 ppm that inhabited the Up1 site.

Arsenic accumulation ranged from 0.45 to 20.4 ppm in samples throughout the watershed. On average, larvae from the Main Branch accumulated the most arsenic (3.1 ppm $\pm$ 0.44 SE). The mean As accumulation for the other three branches of the river were between 2.0 and 2.1 ppm. Out of the twenty insects with the most As accumulation, eleven were collected from the Main branch. One specific location in the Main Branch, Mur2 (Figure 13), yielded larvae with As levels of 20.4, 12.9, 12.3, and 6.7 ppm. Only one other site, Ton1 of the Middle Branch, produced an insect with a higher concentration (6.8 ppm) higher than any measured at Mur2.

Average nickel accumulation was at a maximum of 4.6 ppm $\pm$ 0.37 SE within the Lower Branch. The mean Ni accumulations (ppm $\pm$ SE) for insects collected from the Main, Upper and Middle Branches were 3.4 $\pm$ 0.18, 4.0 $\pm$ 0.30, and 3.8 $\pm$ 0.43, respectively. The Hydropsychidae accumulation of Ni varies significantly between the Lower Branch and at least one other branch. The accumulation of Ni peaked at 8.0 ppm at three locations, Up2, Ton1, and Bell1. The Ton1 site is in a creek of the Middle Branch; the Up2 and Bell1 sites are within the Upper Branch.

Insects collected from the four branches of the Rouge River differed significantly in their average metal content of Al, Mn, Co, Se, As, and Ni. Hydropsychidae from the Lower River Branch had the highest average accumulation of Al, As, Co, Ni, and Mn. The Main Branch had larvae with the highest mean body concentrations of As, and Se. On average, none of the metal accumulation was highest in the Middle River Branch and only less toxic metals (Na, Mg, and K) accumulated to the greatest extent in larvae the Upper Branch.

Land Use Analysis by Branch

In addition to analyzing the metal accumulation in Hydropsychidae collected in River

Branches, the spatial analysis was expanded to include areas with different land uses. Rather than characterizing the metal accumulation by the Upper, Lower, Middle, or Main Branches, the land use was calculated for five distinct drainage areas (Table 8). Each had a unique combination of land cover (Table 8). Residential land cover ranged from 37% to 58%, while land with industrial or commercial applications ranged from 1.5% to 9%. Agricultural land use of the five sub-watersheds was between 0% and 15%. Land that was undeveloped covered 28% to 48% of the areas. The Hydropsychidae collection sites in these areas were used to examine differences in metal accumulation. The sample sizes for the sub-watersheds ranged from 10 to 24. Metal accumulation that varied significantly between at least two of these sub-watersheds included: Al, Cr, Mn, Co, Zn, As, Se, Cd and Pb.

To see if the differences in metal accumulation were linked to land use, the upstream land cover was calculated for all 51 Hydropsychidae collection sites. Land cover was expressed as percentage for four categories: (1) industrial or commercial, (2) residential, (3) agricultural, or (4) undeveloped. The percentage of upstream land cover was referenced to Hydropsychidae metal content to assess correlation. For each metal and land designation, the correlation was quantified as a Pearson coefficient (r). Generally, metal accumulation had a weak correlation to the four categories of land use (Table 7). Arsenic had a weak positive correlation (r: 0.23) to residential land cover. Barium had a weak positive correlation (r: 0.21) to agricultural land use. A weak negative correlation (r: -0.20) was detected between Zn and the percentage of undeveloped upstream land. Undeveloped land cover was also weakly correlated to As (r: -0.21) and Cd (r: 0.20). Only Se had a moderate correlation to any of the four land use categories; it was negatively correlated (r: -0.37) to undeveloped land, and positively correlated (r: 0.315) to upstream residential land cover. No other metal had a moderate or strong correlation to any of the four land use designations.

Land use was positively correlated to larval accumulation of As, Ba, and Se. Drainage areas with more residential land cover were weakly associated with greater As accumulation. Higher Se accumulation was moderately linked to upstream areas with greater percentages of residential land cover. An increase in upstream agricultural land use was linked to elevated larval concentrations of Ba. In contrast, undeveloped upstream land cover had a negative weak correlation to the accumulation of Zn, and As; undeveloped upstream areas had a moderate negative correlation to the concentrations of Se in Hydropsychidae. A negative correlation implies that areas with more undeveloped land tend to have a lower accumulation of Zn, As, and Se.

Temporal Patterns of Metal Accumulation: Watershed

A temporal analysis at the watershed level can be indicative of changes in metal pollution across the region, and the net-effect of many small scale variations, such as changing industrial discharges, runoff, illicit discharges, and land development. The number of Hydropsychidae specimens analyzed for whole-body metal content in 2006, 2008, 2012, and 2015 are 35, 42, 45, and 67 respectively. Accumulation varied significantly between at least two years for 11 metals tested (Table 5).

Of the 11 metals that significantly varied from 2006-2015, only one, cadmium, had the greatest average concentration in 2006. The mean concentration of Cd was highest in 2006 at 0.29 ppm $\pm$ 0.043 SE. The largest recorded level of Cd accumulation was 1.2 ppm and occurred in 2006. Average concentrations of Sr in 2006 were lowest at 19.9 ppm ± 2.1 SE. Larvae from 2006 ranged in Ba concentration from 0.75 to 122 ppm.

Barium varied significantly between 2006 and 2008. The Mean Ba concentration ± SE peaked in 2008 at 60 ppm ± 10.3, and was at its lowest in 2006 at 29 ppm ± 3.5. Of all specimens, the greatest Ba accumulation of 334 ppm occurred in 2008. In this same year, an insect accumulated a minimum of 8.3 ppm Ba. The maximum As concentration of 20.1 ppm was from a Hydropsychidae collected in 2008 (Figure 14). On average, As accumulation was highest in 2008 at 3.2ppm ± 0.52 SE. Lead had the highest mean concentrations of 7.5 ± 2.9 ppm in 2008. This same year was also when volunteers collected the larva with single greatest contamination of 127 ppm Pb within the Upper Branch (Field ID: Up2) (Figure 15).

Lead concentrations varied significantly between 2008 and 2012 with respective averages ± SE of 7.5 ppm ± 2.9 and 3.7 ppm ± 0.71. The ranges for the same corresponding years were 1.8 – 126 ppm (2008) and 0.8 – 45 ppm (2012). Insect larvae from 2012 yielded the highest average strontium accumulation of 32.2 ppm ± 3.7 SE. Larvae collected in 2012 had the lowest average Cd accumulation of 0.15 ppm ± 0.015 SE. The lowest mean As value of 1.6 ppm ± 0.11 came from 2012.

A specimen from 2015 accumulated 2029 ppm of Al, the highest concentration measured in the watershed. The 2015 samples also had the highest average Al concentration (743 ppm + 93 SE) with a range of 125 – 5224 ppm. Cobalt also accumulated to the greatest extent in 2015. The lowest averages of 3.7 ± 0.71 ppm Pb came from the 2015 sampling. The mean accumulation of manganese (2122 ppm + 246 SE) reached a maximum in 2015. One Hydropsychidae reached a dry body weight concentration of 10,126 ppm Mn.

In 2015, approximately 16% of larvae collected recorded higher concentrations of Mn then any insect from 2006, 2008, or 2012. In 2015, average larval Co concentrations $\pm$ SE were 2.37 ppm $\pm$ 0.31 compared to 1.16 ppm $\pm$ 0.09, 1.60 ppm $\pm$ 0.11, and 1.15 ppm $\pm$ 0.089 in 2006, 2008, and 2012, respectively. Ten percent of samples collected in 2015 had greater Co concentrations than any larvae from the other three years. From all four sampling years, As accumulation throughout the watershed ranged from 0.44 to 20.1 ppm. The respective ranges of Sr for 2006, 2008, 2012, and 2015 were 5.3 – 64.4, 9.4 – 84.0, 6.9 – 158, and 8.8 – 172 ppm. The larvae collected in 2006, 2008 and 2012 ranged from 68 – 1766 ppm Al. Accumulation of Co ranged from 0.67 – 11.6 ppm for all specimens.

Comparison with Historic Levels of Metals in Sediment

In addition to comparing metal accumulation from different sampling years, historic metal content of the Rouge River bed sediment was referenced to the metal concentrations of Hydropsychidae. Murray et al. (1997) analyzed the bed sediment of the river throughout the watershed. I paired the metal accumulation data with common sampling sites of the bed sediment. Sites were considered common if they fell within 0.1 mile upstream or downstream of one another. By pairing the metal concentrations of the bed sediment and net-spinning caddisflies, I was able to calculate a Pearson correlation coefficient (r) to see if the historic points of metal contamination were linked to accumulation in larvae from this study. Metals evaluated for correlation included Pb, Cr, and Cu. In the bed sediment lead concentrations ranged from 5 to 380 ppm dry weight (Murray et al., 1997). Chromium was between 4 and 50 ppm in the sediment, while Cu ranged from 80 to 1600 ppm (Murray et al., 1997). In 1997, the average concentrations of Pb, Cr, and Cu in the sediment were 56.2, 17.0, and 371.7 ppm. At these same locations the accumulation of Pb, Cr, and Cu from the 2006-2015 Hydropsychidae samplings were 6.4, 5.5, and 3.8 ppm. A correlation coefficient was calculated for Pb (n: 74), Cr (n: 36), and Cu (n: 12). The correlation coefficients (r) for Pb, Cr, and Cu, were -0.06, 0.18, and 0.03 respectively. The 1997 bed sediment content of Cr had a weak positive correlation to accumulation in Hydropsychidae. The locations with increased concentrations of Cr in the sediment in 1997 generally had net-spinning caddisflies with elevated Cr accumulation in 2006-2015. No such pattern was detected for Pb and Cu. Lead and Cu did not show a correlation between concentrations of the 1997 bed sediment and Hydropsychidae from 2006-2015. Here, the Hydropsychidae accumulation of Pb and Cu was not linked to the concentration of these metals in sediment measure in 1997.

Within Branch Analysis

Main Branch

This portion of the study determined the concentration of metals from 56 net-spinning caddisflies from the Main Branch of the Rouge River. Respective sample sizes for the year of 2006, 2008, 2012, and 2015 were 10, 14, 20, and 12. Within the Main Branch of the Rouge River, Hydropsychidae accumulation differed significantly between at least two collection years (2006, 2008, 2012, and 2015) for eleven different metals (Table 6). Average Cr concentration in 2008 were the greatest at 5.24 ppm $\pm$ 0.49 SE. Five of the highest ten concentrations of Cr within the caddisflies were from 2008.

Those ten larvae ranged from 6.09 ppm (Field ID: Main3) to 8.69 ppm (Field ID: Nott). Other average metal concentrations that reached relative maximums in 2008 include: Ni, Cu, As, Ba, Pb and Fe. Mean Ni accumulation reached a maximum of 4.3 ppm $\pm$ 0.45 SE in 2008 compared to the lowest average of 2.5 ppm $\pm$ 0.15 in 2015. A single insect from Nottingham

Creek (Field ID: Nott) accumulated the most Ni with 7.9 ppm by body mass. Unpolluted rivers have between 0.1-10 ppm dissolved Ni compared to contaminated rivers with dissolved Ni concentrations between 50 and 2000 ppm (Chau and Kulikovsky-Cordeiro, 1995). Schubauer-Berigan et al. (1993) reported lethality occurs in sensitive aquatic species at 11 ppm. Although the average Ni concentrations differed significantly, the relatively low concentrations accumulated throughout the Main Branch do not indicate aquatic populations are being substantially depressed by this particular metal.

Average Cu accumulation ranged from 37.6 ppm $\pm$ 3.3 SE in 2008 to 28.3 $\pm$ 2.1 in 2015. Copper levels ranged from 18 to 71 ppm within individual larva of the Main Branch. Three of the top five concentrations of Cu and As measured came from Hydropsychidae captured during 2008. On average, 2008 insects had As concentrations of 5.4 $\pm$ 1.4 SE with concentrations reaching as high as 20 ppm. Two 2008 specimens from Murphy Creek (Field ID: Mur2) (Figure 14) accumulated the greatest concentrations. In fact, the four highest recorded values of As in the Main Branch came from the same location (Mur2) (Figure 14). The 2015 collections produced these other two insects from Mur2 with As concentrations of 12.3 and 6.7 ppm. Larvae from 2008 held the greatest mean concentration of barium (53.5 ppm $\pm$ 10.3 SE) and lead (4.8 ppm $\pm$ 0.46 SE). Barium ranged from 6.0 to 329 ppm (Field ID: Mur2) (Figure 16). Lead ranged from 0.95 to 8.8 ppm (Field ID: Nott) (Figure 16).

Upper Branch

The accumulation of As varied significantly ($p = 0.009$) across the sampling years (2006, 2008, 2012, 2015) in the Upper Branch of the Rouge River. Eight samples were analyzed from 2006, while the number (n) of Caddisfly larvae from 2008 and 2012 were both 6. There were 10 samples from 2015. The collection locations varied from year to year. Sites were coded by a field identification (Figure 5). The 2006 specimens were collected at the field locations of: Min3, Up2, Up1 and See1. In 2008, the locations included Up1, Up2, and Bell3. The field identification sites for 2012 were Up1, Up2, and Min2. Lastly, the 2015 metal accumulation was represented by larvae from Bell2, Min3, Up1, Min2, and Bell1. The respective As means, ppm $\pm$ standard error (SE), for the Upper Branch locations of 2006 and 2008 were 2.3 ppm $\pm$ 0.57 and 2.2 ppm $\pm$ 0.32. Samples from 2015 yielded the highest average As concentrations of 2.6 ppm with a standard error of 0.28. In contrast, the caddisflies averaged only 1.0 ppm As $\pm$ 0.18 SE in 2012. The As highest accumulation of 6.6 ppm occurred in 2006 from a larvae recovered from Minnow Pond (Field ID: Min3) (Figure14). Within the Upper Branch from 2018-2015, Hydropsychidae accumulated significantly different mean concentrations of Al, Co, Ba, and Fe. Average As and Co concentrations reached a maximum in 2015 at 898 ppm $\pm$ 142 SE and 2.3 $\pm$ 0.35 respectively. The lowest recorded averages occurred in 2012 at 324 ppm Al $\pm$ 92 SE and 0.75 ppm Co $\pm$ 0 .11. Average Ba concentrations increased each year. At least two of the years varied significantly by Ba concentration at the p level of 0.013. Barium concentrations (mean $\pm$ SD) for 2006 were 27 ppm $\pm$ 4.6. Larvae from 2015 had mean body concentrations of 65 ppm Ba $\pm$ 12.2. One larva collected at Min3 showed relatively high levels of Ba at 169 ppm.

Middle Branch

Net-spinning caddisflies (Hydropsychidae) were collected from 20 different locations within the Middle Branch of the Rouge River. Collection years in this analysis include 2006 (n: 11), 2008 (n: 20), 2012 (n: 16), and 2015 (n: 26). A comparison of average metal concentrations during these time periods resulted in significant differences between at least two years with respect to Mn, As, Se, Sr, Cd, Ba, Na, Ca, and Fe. Manganese accumulation showed significant variation over time (p: 0.009). Average Mn accumulation was the highest in 2015, at 2026 ppm

± 351 SE. In contrast, larvae from 2012 accumulated the lowest average concentration of 921 ppm ± 77 SE. Ten of the twelve highest readings were from 2015. Manganese concentrations were the highest in 2015 at 8412 ppm from a Tonquish Creek specimen (Field ID: Ton1) (Figure 12). The respective concentrations (average ± SE) for 2006 and 2008 were 997 ppm ± 211 and 1019 ppm ± 10.

Caddisfly larvae collected from the Middle Branch in 2015 had the highest Ba concentrations (58.0 ppm ± 7.4 SE), just as they did in the Upper Branch. Similarly, average Ba values were lowest in 2006 for both the Middle and Upper Branches. In 2006, the mean accumulation was 31.1 ppm Ba ± 4.3 SE in the Middle Branch. Two samples collected in 2015 from Tonquish Creek (Field ID: Ton1) had the greatest Ba concentrations of 174.4 and 147.1 ppm (Figure 16). This same location yielded maximum levels of As and Mn in 2015 as well. From the four sampling years, only a single caddisfly larvae of the Middle Branch accumulated levels of selenium at potentially hazardous levels of 11.5 ppm. This specimen was collected in 2012 from Johnson Creek (Field ID: John5).

As with the Upper Branch, average As accumulation in the Middle Branch significantly differed (p: 0.009) between at least two of the spring collections. Specifically, the largest difference occurred between 2006 (1.2 ppm As ± 0.13 SE) and 2008 (2.2 ppm As ± 0.20). Samples analyzed from 2015 also showed relatively higher As concentrations of 2.0 ppm ± 0.28 SE. Similar to Mn, some of the highest As concentrations (6.8 and 6.1 ppm) came from 2015 larvae collected from Tonquish Creek (Field ID: Ton1). The toxic effects of strontium are not well understood. No established water quality guidelines exist to protect aquatic wildlife. Thus, evaluating the concentration within the larvae of the Rouge River is difficult. Nevertheless, within the Middle Branch, accumulation varied significantly (p: 0.013) between samples collected in 2006 and 2012. Average concentrations in 2006 were 17.4 ppm ± 2.2 SE and the larvae in 2012 had mean concentrations of 30.0 ppm ± 3.9 SE. Two samples taken from Bishop Creek in 2012 (Field ID: Bish2) had the highest Sr levels of 56.5 and 52 ppm. In 2006, mean Cd accumulation was significantly higher (p: 0.008) in the Middle Branch compared to 2012. Averages ± SE for the corresponding years were 0.32 ppm ± 0.93 and 0.13 ppm ± 0.016. A Hydropsychidae larva from the 2006 collection in Johnson Creek (Field ID: John2) had Cd levels of 1.2 ppm, more than two fold higher than any other sample from the Middle Branch.

Lower Branch

For the Lower Rouge, the analysis of the four sampling years (2006, 2008, 2012, and 2015) is relatively limited due to the small sample sizes of 2008 and 2012. Only two insects were collected during both of those years. The 2008 samples were collected from Fowler Creek (Field ID: Fowl 2) and the 2012 samples were taken from Fellows Creek (Field ID: Fel1). Volunteers collected more specimens in 2006 (n: 6) and 2015 (n: 13). Annual collections vary according to the number and locations of volunteer groups working with Friends of the Rouge, a local river advocacy group. Although the sparse sample size may not be representative of the temporal changes, a few metals significantly varied between at least two years. Statistical analysis revealed significant differences in the means of 0.05 for Sr (p: 0.046), Ba (p: 0.0007), Na (p: 0.015), and K (p: 0.0009).

Average Sr accumulations reached a maximum of 60.7 ppm ± 23.2 SE in 2008. The respective mean concentrations ± SE in 2006, 2012, and 2015 were 17.1 ppm ± 3.2, 32.4 ppm ± 5.6, and 28.1 ppm ± 4.2. None of the 6 insects collected in 2006 had Sr concentrations greater than 50 ppm. On average, 2006 larvae had 31.4 ppm ± 3.5 SE by dry weight. In 2015, the mean Sr concentration for the Lower Rouge (n: 13) was 47.2 ppm ± 7.7 SE. As with Sr, 2008 samples had the highest levels of Ba with an average of 325 ppm $\pm$ 8.8 SE. These two caddisfly larvae were from a single location (Field ID: Fowl2).

Markers of Metal Accumulation

A darkening of the tracheal gills and papillae was linked to a host of contaminants, including NaCl (road salt), nitrates, phosphates, K, Mg and ammonia. Specimens from the Rouge River were tested for a significant difference between the mean body metal concentration of larva with and without protruded papillae (Figure 10). A Student's T-test revealed no significant difference between these two groups of larvae and nearly all the metals tested (Appendix 4). Strontium was the exception to this trend (p: 0.05); larvae with protruded papillae had a lower average metal accumulation of 2.0 ppm compared to larvae without this morphological marker with a mean of 2.8 ppm Sr. The T-test was conducted as a two-tailed calculation assuming unequal variance. For the 18 metals analyzed, the p values ranged from 0.06 to 0.95. The sample size was 10 for the larvae with protruded anal papillae. This group of ten was compared to a group of 172 individuals without the protrusion. Thus, metals alone may not be responsible for this particular morphological marker of pollutants.

A reduction of benthic biodiversity has also been associated with elevated levels of metals. To examine biodiversity as an indicator of metal pollution a Pearson's correlation test was conducted for Hydropsychidae metal accumulation with a biodiversity score (SQI) and benthic organisms richness, number of different orders (Appendix 3). Both the richness and biodiversity values were provided by Friends of the Rouge. The biodiversity scores had a weak negative correlation to the Hydropsychidae metal accumulation of Cr, (r: -0.140), Zn (-0.132), and Pb (-0.166). The number of different taxa (order) present had a small negative correlation to the accumulation of Cr (r: -0.106), Ni (r: -0.108), and Zn (r: -0.137). The negative correlations between the biodiversity measures (SQI and number of taxa) implies that some sites with higher levels of metal accumulation were associated with lower biodiversity measures. The accumulation of Mn, Ba, and Cd had a weak positive correlation to both the biodiversity scores and taxa richness (Table 9). The other metals measured in this study had no correlation to these two measures of the benthic community (Table 9).

Chapter 5: Discussion and Conclusions

Indicators of Metal Accumulation

Biological indicators and markers have the potential to help monitor the environment, track remediation, and assess threats to an ecosystem. Hydropsychidae larvae are suitable indicators of toxic metal contamination that complement other methods of water quality assessment. Hydropsychidae can tolerate elevated levels of metals and can assist in identifying wide ranging toxic effects. The variable metal concentrations accumulated within the larvae illuminate site specific contamination. Such contamination is deleterious to the exposed biota. Significant fluctuations in metal accumulation measured in this study demonstrate the need for widespread monitoring. Sampling and tissue analysis across the watershed is imperative in limiting the impact of human actions.

Biological markers reflect an interaction between a hazard and a biological system. For example, measurements of chemical, physical, and biological factors are used as markers, in addition to changes in behavior. The WHO places biomarkers into the following three categories: exposure, effect, and susceptibility (Poonam and Jaffery, 2004). A biomarker of exposure is a measurable factor that signifies an interaction with a foreign substance. An effect is not only an indicator of contact, but also of damage to tissue.

Identifying a hazard is an integral part of analyzing an environmental risk. In addition to identification, the effect and potential exposure of a hazard must also be considered. In contrast to humans, harmful outcomes in wild populations are oftentimes detected after long periods of exposure (Oost et al., 2003). Bioaccumulation occurs when persistent chemicals enter an organism regularly. Such chemicals can enter an organism as a result of contaminated food, the ingestion of suspended particles, or be absorbed by the gills or skin. Aquatic ecosystems are especially vulnerable because they are a long term sink for many anthropogenic contaminants (Oost et al., 2003). Agricultural and industrial wastes have a pathway to rivers, oceans, lakes, and groundwater through atmospheric deposition, discharge, and runoff. This increases the likelihood that multiple stressors can contribute to the degradation of an ecosystem. The presence of multiple stressors can confound the toxic analysis of a particular substance. Many attributes of an organism and the environment may influence the accumulation of pollutants. Some of these confounding factors include: sex, migratory patterns, water temperature, population density, and the distribution of the pollution (Oost et al., 2003). Regular sampling and analysis of a variety of biological indicators is essential to understanding the hazards that may degenerate an ecosystem.

Kakkar and Farhat (2005) classify chemical markers for accumulated metals into four categories: (1) Molecular lesions are damaging changes to biological molecules such as amino acids, enzymes, and genetic material; (2) Exogenous chemicals have an origin from outside of the organism; (3) Endogenous molecules are produced by the body in response to a toxin. For example, porphyrin molecule ratios change in response to Pb and other metals. Porphoryins are biosynthesized molecules that can exchange metal ions with ligands; and (4) Cellular or tissue damage, such as sperm motility or blood cell counts, can also signal toxic chemical exposure. In addition to chemical analysis, assessing the stream biodiversity or lack pollutant sensitive invertebrate species can indicate metal contamination. Elevated concentrations of metal have been linked to the absence of species or a reduction in taxa. Biomonitoring based on the absence of species or taxa may not be informative in terms of sub-lethal concentrations of a specific pollutant. Identifying behavioral or morphological markers can provide a non-invasive and earlier detection of environmental toxins. Easily tagging individuals exposed to sub-lethal concentration would enhance the opportunity to examine the cellular or molecular mechanisms of toxicity (Habib et al., 2016). Habib et al. (2016) established behavioral markers for toxic metals in a snail species, *B. alexandrina*. Exposure to Cd and Mg inhibited locomotion, feeding, and the snail's attachment to a surface. These particular changes in behavior show promise in identifying chronic and acute metal exposure. Further testing with other metals would be beneficial to extend the utility of the behavioral marker for metal contamination in aquatic environments. Behavioral markers for a toxin observed in the lab need to be corroborated with findings from organisms in the wild. Non-invasive markers in behavior and morphology could be associated with many environmental contaminants. Monitoring the toxic effects of metals is becoming increasingly important with industrialization. As human activities concentrate metals in the environment, the effect on terrestrial and aquatic species must be examined.

Benthic invertebrate, such as caddisflies, are the best measure of the impact of metals because these organisms interface with the water, soil, and higher levels of the trophic system. They are a common prey item for fish which carry energy and contaminants to higher levels of the food chain. Hydropsychidae have been commonly used as bioindicators in other studies. Tissue sampling can play a vital role in complementing the physical and chemical parameters used to characterize an aquatic ecosystem.

Multiple studies have linked elevated quantities of metals to the morphological deformation of the anal papillae in Hydropsychidae. Identifying exposure or contamination through chemical means involves gathering a tissue sample from the environment followed by chemical analysis. Vuori and Kukkonen (1996) along with Tszydel et al. (2015) reported deformation of the ion regulating anal papillae in Hydropsychidae exposed to some pollutants. Both studies reported a correlation between elevated levels of metal accumulation and protruded papillae (Figure 10). Here, we did not find a correlation between larval metal accumulation and a protrusion of the anal papillae.

Working towards better understanding a biological marker for contamination will enhance predictions about the extent of pollution and long term impact on the ecosystem. Examining metal accumulation in caddisflies helps establish reference data to assist in distinguishing between background or natural concentrations, and those elevated by an anthropogenic source. Evaluating the toxicity, distribution, and severity of metal pollution can help shape policy, land use, and remedial action. Even though this study did not find a morphological marker for pollutant in the Hydropsychidae family of the Rouge River Watershed, an expedited discovery of metal pollution could limit toxic effects by addressing point sources.

Although this study did not find an association between protruded anal papillae and the levels of metals in Hydropsychidae, a simple indicator would assist in stream monitoring. Biodiversity surveys within the aquatic invertebrate community are commonly used to assess water quality. However, a whole host of contaminants and physical water properties can reduce biodiversity. This was exemplified by the correlations between Hydropsychidae metal accumulation and the biodiversity measures taken from the Friends of the Rouge. The weak negative correlations between Cr, Zn, and Pb accumulation to biodiversity measures may indicate the metals are restructuring benthic communities at some sites. However, a weak correlation also indicates that other factors are likely influencing the benthos. The concentration of some metals (Mn, Ba, and Cu) of Hydropsychidae were even positively correlated to the biodiversity scores and number of taxa at certain locations in the river. These metals are known toxins, and the positive correlations are likely due to other characteristics of the stream influencing the benthic community. Some of those factors could include organic pollutants, flashiness, sedimentation, dissolved oxygen levels, and biological oxygen demand. Metal pollution can lower benthic biodiversity, but it is not an ideal indicator of metal contamination because of all the other stressors that could depress or extirpate benthic populations. The regular analysis of tissue is necessary to evaluate metal pollution because so many other contaminants and stressors can confound other indicators such as community measures, morphological markers, or behavioral markers.

Contaminant specific markers would certainly help stakeholders and watershed managers make more informed decisions to preserve stream quality. Qu et al. (2010) found a correlation between metal pollution and an increase in the ratio of predatory macroinvertebrates compared to those that feed by scraping and grazing. Investigating this correlation would be a viable and gainful follow up study. Friends of the Rouge has preserved invertebrate populations that correspond to the Hydropsychidae with known body concentrations of metal. Calculating the ratio is relatively simple and could be used to direct more expensive and sophisticated analysis of tissues for metal concentrations.

Relative Metal Accumulation

Compared to other watersheds, the Rouge River has relatively low average metal accumulation in net-spinning caddisflies. Limited metal accumulation in Hydropsychidae of urban watersheds makes comparisons difficult. Table 3 compares the accumulation of 9 different metals in the Hydropsychidae larvae captured from the Rouge to rivers in Poland and Spain. Although other watersheds do not align as well to the characteristics of the Rouge River watershed, these Hydropsychidae values can at the minimum provide context for metal pollution in this family of insects. Some of the sampling points are associated with known sources of pollution, and others are from relatively undisturbed portions of the river.

A watershed in Lodz, Poland is the most similar and appropriate watershed for comparison to the Rouge River watershed. Tsyzdel et al. (2015) collected Hydropsychidae from 9 sites in an urban watershed (Lotz, Poland) with a population of about 740,000 people. All 9 sites were located in first or second order streams. As with this study, almost all of the insects were collected from the headwaters. On average most of the Hydropsychidae metal concentrations were lower from the Rouge River larvae compared to those from Lodz (Table 3). For example, the Lodz insects had average Fe, and Zn accumulations of 4,500 and 300 ppm while, specimens from the Rouge River had 270 and 160 ppm respectively. The Rouge River also yielded lower averages of Pb (4.7 ppm) and Cd (4.7 ppm) relative to the means of 75 ppm Pb and 7.5 ppm Cd in this similar urban watershed. These same two urban watersheds produced Hydropsychidae smaller differences in average Cu and Cr accumulation. The Rouge River watershed had slightly lower means for Cu, 33.2 ppm compared to 37 ppm. The Cr average 4.7 ppm was also lower than the 5.3 ppm Cr recorded in the Lodz specimens. Samples from Lotz had substantially higher levels of Cu, Fe, Zn and Pb. For example, the average Pb accumulation in the Ner and Bzura rivers of Lotz was 75 ppm compared to only 4.7 ppm in the Rouge. Similarly, these rivers in Poland were home to larvae with an average of 7.5 ppm Cd, whereas Hydropsychidae that inhabited the Rouge had a mean of 0.2 ppm. Generally, the average metal uptake recorded from the Rouge River is consistent with areas of moderate or mild contamination with the exception of manganese. Average Mn concentrations in Rouge were

1,368 ppm compared to 477 ppm at a site downstream of a known chemical dump (Cid, 2010), and 140 ppm at a relatively undisturbed sampling location (Cid, 2010).

Generally, the larvae from the Rouge had lower levels of some metals (Zn, Cd, As, and Ni) compared to three other sites associated with mining, industry, and agriculture. However, this was not the case for Manganese. Although, accumulation data is limited, elevated freshwater levels of Mn are associated with depressed growth and reproduction in some freshwater fish and invertebrates (Reimer, 1999). Hydropsychidae from the Rouge River had a mean concentration of 1,368 ppm Mn compared to 477 ppm at a site associated with chemical dumping (Cid et al., 2010). Benthic invertebrates collected in an urban center (Sao Carlos) of Brazil, also had much lower mean Mn concentrations of about 83 ppm (Chiba, 2011). The samples from the Rouge River were collected from the headwaters and might not be indicative of areas of greater contamination in higher order streams. Many of the industrialized areas that likely contribute more to the concentration of metals are also located near the higher order streams in the Rouge River watershed.

Temporal Trends in Metal Accumulation

Over the four sampling years, 2006-2015, the watershed produced significant differences in the mean accumulation of Pb, Al, As, Cd, Ba, and Mn. Insects from 2008 had the highest average lead values of 7.5 $\pm$ 2.9 SE. The maximum accumulation in any insect of 127 ppm also came from an insect collected in 2008. I measured the lowest average Pb (3.7 + 0.71 ppm) concentration in insects from 2015. The watershed showed relatively low levels of cadmium with mean values peaking in 2006 at 0.29 ppm $\pm$ 0.043 SE. A 2006 insect had the single highest Cd accumulation of 1.2 ppm.

In addition to differences in metal accumulation at watershed level, within the River Branches some metal concentrations varied as well. Between 2006 and 2015 the mean accumulation of Pb, Mn, Cr, As, Cd and Cr varied significantly within the Main River Branch. From 2006-2015 Hydropsychidae of the Upper Branch significantly differed in their mean concentration of As, Al, Co, and Ba. In the Middle Branch of the Rouge River, average accumulations of As, Cd, Se, Sr and Ba varied significantly between at least two of the sampling years. Caddisfly larvae collected from the Middle Branch in 2015 had the highest average Ba concentrations of 58.0 ppm $\pm$ 7.4 SE. In 2006, the mean Ba accumulation was the lowest at 31.1 ppm $\pm$ 4.3 SE in the Middle Branch. Barium concentrations in water and sediment can vary greatly with local geology. Lethal concentrations in crayfish were reported at 31 to 39 ppm by Boutet and Chaisemartin (1973). The differences in Hydropsychidae metal concentration from 2006-2015 illustrate the changing conditions and health of the Rouge River watershed.

Many metals reached a relative high in 2008. Within the watershed average accumulations were highest in 2008 for As, Ba, and Pb. In 2008 within the Middle Branch, Cr, Ni, Cu, As, Ba, and Pb had the highest averages of all four sampling years. A possible contributor to these averages could be the amount of precipitation and snowfall. As snowfall increases, so does the use of salt and sand to treat roadways and walkways. Sand and salt can contain trace amount of metals that will eventually be swept into the river by runoff. In 2008, two National Weather Service (NWS) field locations recorded an average of 55 inches of snowfall in the Rouge River Watershed. The same field locations measured 24, 38, and 35 inches of snow in 2006, 2012, and 2015 respectively. In terms of total precipitation, the NWS reported the averages for heated gauges at 39, 34, 29, and 30 inches for 2006, 2008, 2012, and 2015 respectively. The heated gauges melt ice, sleet, and snow to measure total precipitation.

The higher snowfall in 2008 could have indirectly increased metal loading into the river as more salt and sand reached the river through runoff. Although, sodium concentrations reached a maximum in 2015 at 8,400 ppm compared to 4,000 ppm in 2008. Only Cd had the highest average accumulation in 2006, which had the greatest total precipitation. Total precipitation may have less of a correlation than total snowfall with respect to loading metals into the river.

Even though metals are persistent, they can be transported and bound in different inorganic and organic forms. The transport and transformation of metals would influence their uptake by living organisms. The concentration of Cr in bed sediment from 1997 showed a weak positive correlation (r: 0.18) to the Cr accumulation in net-spinning caddisflies. No correlation was detected for Pb (r: -0.06) and Cu (r: 0.03) between the concentrations in the bed sediment and Hydropsychidae. Net-spinning caddisflies are known to take in metals from sediment. The bed sediment composition has likely changed between 1997 and 2006. This may have contributed to the absence of a moderate or strong correlation in this case. Such a change in the conditions of the river underscores the need for regular tissue analysis to monitor the health of a river and watershed. To help interpret the impact of tissue analysis, toxicity testing specific to Hydropsychidae would be helpful in judging the effect of metal accumulation on rivers. Ideally, toxicity testing would include standards expressed as metal concentrations in waters and tissues. Using tissue and water toxicity standards would allow stakeholders to better assess the negative impacts of metal contamination.

Spatial Trends in Metal Accumulation

A variety of factors can influence the distribution of metals in the environment. Some of these factors include land use, land development, and sediment composition. Economic development can be tied to metal contamination. The combustion of coal increases with development and is a major source of Pb and As in sediments. The resulting aerosols from burning coal can precipitate and supply a significant quantity of Pb and As to sediments. For example, development over two decades along the Pearl River in China, paralleled an increase from approximately 35 to 55 ppm in Pb concentrations within the sediments of the river's estuary (Li et al., 2000). Stable isotope studies support that the deposition from the combustion of coal had a likely anthropogenic source. Researchers have determined the sediments and coal have similar ratios of $^{206}Pb/^{207}Pb$ of about 1.181 and 1.187 respectively (Li et al., 2000). As with Pb, other metals are likely connected to urbanized areas such as the Rouge River Watershed. For example, wastewater discharge is the primary source of global freshwater manganese contamination (Reimer, 1999). Elevated metal concentrations in the Rouge River Watershed were linked to increases in urbanization and industrialization by Murray et al. (2004).

This study found that most metals had a weak correlation to upstream land areas in the four following categories: industrial or commercial, residential, agriculture, or undeveloped. Residential land cover had a weak positive association (r: 0.23) with Hydropsychidae As accumulation. Agricultural land cover had a weak positive correlation (r: 0.20) to Ba accumulation. Undeveloped land had weak negative correlations to the accumulation of Zn (r: -0.20), As (r: -0.21), and Cd (r: 0.20). Upstream residential land cover had a moderately strong correlation to Se (r: 0.315); Se had a moderately strong negative correlation (-0.37) to undeveloped areas. No other metal accumulation had moderate or strong correlations to the four land use categories. Certain industries such as refineries, disposal site, plating facilities, battery manufacturers and many others are known polluters of metals. The industrial or commercial category may be too general to capture the effect of such operations. This could account for the lack of a moderate or strong correlation between industrial or commercial land use and the

Hydropsychidae metal accumulation. Perhaps an analysis that references more specific industrial activities to metal accumulation would result in more detailed geographic findings describing the connection between land use and metal accumulation.

The metal accumulation across the watershed varied significantly across the different branches of the Rouge River. Between the branches of the River, I measured significantly different mean accumulation of Mn, Al, Co, Ni, and As. Hydropsychidae contained As levels between 0.45 to 20.4 ppm. Average nickel accumulation was at a maximum of 4.6 ppm $\pm$ 0.37 SE within the Lower Branch. The mean Ni accumulations (ppm $\pm$ SE) for insects collected from the Main, Upper and Middle Branches were 3.4, 4.0, and 3.8, respectively. The Hydropsychidae accumulation of Ni varies significantly between the Lower Rouge and at least one other branch. The accumulation of Ni peaked at 8.0 ppm at three locations, Up2, Ton1, and Bell1. The Ton1 site is in a creek of the Middle Branch; the Up2 and Bell1 sites are within the Upper Branch. A host of aquatic invertebrates are used to assess water quality. Aquatic insects near one of the largest global Ni deposits in Sudbury, Ontario had mean Ni concentrations of 22 ppm (Chau and Kulikovsky-Cordeiro, 1995). Benthic invertebrate populations thrive at much higher concentrations, greater than 100 ppm. However, some fish and birds species experience teratogenic effects and population decline as dietary Se levels exceed 3 to 11 ppm (DeBruyn and Chapman, 2007). Teratogenic toxins interfered with the development of an embryo.

Arsenic accumulation ranged from 0.45 to 20.4 ppm in samples throughout the watershed. On average, larvae from the Main Branch accumulated the most arsenic (3.1 ppm $\pm$ 0.44 SE). Mean concentrations of As (3.1 ppm $\pm$ 0.44 SE) were the greatest in insects from the Main Branch relative to the other three branches with averages between 2.0 and 2.1 ppm. Stonefly larvae have an LC50 of about 1 ppm As in water (Eisler, 1988). The accumulation of

As in stoneflies can reach 130 times the concentration of As in streams (Spehar et al., 1980). Stoneflies are considered more sensitive to pollutants, and do not inhabit degraded streams like net-spinning caddisflies. The accumulation of As in Hydropsychidae does not indicate this metal is depressing benthic invertebrate populations.

Average Al concentrations were at a maximum of 799 ppm in the Lower Rouge River. The toxicity of Al is dependent on pH. In acidic waters (pH <5.5), the metal is more available to living organisms, and thus more toxic (Sparling and Lowe, 1996). In streams that have a pH that exceeds 5.5, Al is not considered toxic. The surface waters of the Rouge River and Southeastern Michigan are not acidified. Aluminum is not likely degrading the benthic invertebrates of the Rouge River.

Proximity to the headwaters or stream order could influence the metal availability in a river. Within the Rouge River Watershed, metal accumulation might increase as you move away from the headwaters. The sampling of larvae did not include downstream locations near the Watershed's more industrialized area, especially the last 5.5 miles before the confluence of the Rouge and Detroit Rivers. This was one limitation of the study. Collecting and analyzing Hydropsychidae from this area would add to this characterization of metal accumulation. Metal concentrations may increase or decrease in the downstream direction. In neighboring rivers in China, Cr, Cu, Ni and Pb sediment concentrations increase in the upstream direction in one river and increased in the downstream direction in the other (Gao and Chen, 2012). The changing concentrations of were attributed to different land uses in the two watersheds (Gao and Chen, 2012).

Industrial land use is linked to elevated concentrations of metals in the environment. The Upper sub-watershed has the lowest amount of industrialization at 4% land cover (Rouge River

Watershed Management Plan, 2012). The Main, Lower, and Middle sub-watersheds have industrialized land areas of 7%, 9% and 10%, respectively (Rouge River Watershed Management Plan, 2012). With the Upper Branch having less industrial area, it may be less prone to metal accumulation. This was not always true in the analysis at the watershed level. For example, the average concentration of Al was 627 in the Upper Branch compared to 547 ppm in the Middle Branch. The Middle Branch has the most industrialization but insects from this portion of the River did not have a maximum average concentration for any of the metals that significantly varied. The sampling of Hydropsychidae was limited to the headwaters. An analysis including the lower portions of the river may have provided a more detailed patterns of metal accumulation and land use. Trends in land use and metal concentration are established in many other studies (Sakan et al., 2015; Gao and Chen, 2012; Weigo et al., 2009).

The analysis Hydropsychidae metal accumulation could be applied to other watersheds. Further toxicity testing and studies on land use and metal accumulation would advance the use of net-spinning caddisflies as a biological monitor. Routine monitoring could assist in linking metal contamination to sources such as illicit discharges or changes in land use. For example, although Pb concentrations were on average relatively low, one site produced an insect with 126.8 ppm. Further investigation of a particular location could assist in locating a point source of pollution. Sediment analysis, community measures, or morphological markers can be misleading indicators of the negative effects of metals on freshwater organisms. Metal uptake from sediments is highly variable in different species and sediment fractions. Community measures and morphological markers are broad indicators of stressors, and are not specific to metals. The analysis of tissues is the most direct and informative indicator of community degradation due to metals. Many attributes make Hydropsychidae excellent bio-indicators. The larvae generally remain in one location unless disturbed. They are in contact with surface water and sediment and persist in degraded stream. Hydropsychidae are a food source for consumers, and thus tissue concentrations can indicate a threat to an entire community. Hydropsychidae can be used to evaluate metal accumulation because of their interface with benthic sediments and ability to persist in degraded streams. Net-spinning caddisflies can be used in routine stream monitoring to identify sites with elevated metals, especially in urbanized watersheds. Urbanized watersheds can have sources and paths for metals to contaminate rivers that change through time and geographic space. Corrective operations and recommended use impairment cannot take place without identifying degraded components of an ecosystem.

This study found that Hydropsychidae metal concentration significantly varied through time and space. Metal accumulation that significantly varied in different branches and sub-watersheds included Al, Zn, As, Cd, Pb, Mn and Co. From 2006 to 2015 Hydropsychidae body concentrations varied for Al, Cr, Mn, Ni, Cu, As, Cd, and Ba across the entire Rouge River Watershed or within a River branch. Metal accumulation in net-spinning caddisfly tissue changes with time and geographic location. Many of the metals are toxic, and have the potential to degrade aquatic and riparian ecosystems. For example, certain aquatic invertebrates have reduced survival and growth at Cr concentrations greater than 10 ppm (Outridge and Scheuhammer, 1993). For consumers such as fish, birds, and mammals dietary Cr concentrations greater than 10 ppm pose a health and reproductive risk (Outridge and Scheuhammer, 1993). Chromium concentrations reached a potentially hazardous level of 16.3 ppm in Hydropsychidae from the Rouge River. On average, the larval Cr concentration was 4.3 ppm and not indicative of widespread contamination. However, average Mn accumulation was elevated compared to other watersheds. Freshwater Mn contamination has been associated with depressed growth and reproduction in some freshwater fish and invertebrates (Reimer, 1999). Hydropsychidae tissue analysis can be used to identify potential threats to freshwater aquatic and riparian ecosystems from elevated levels of Mn, Cr, Pb and other toxic metals. Metals are persistent and can permeate through aquatic and terrestrial trophic systems. Tissue samples are a direct measure of metal accumulation. Considering the high toxicity of metals and their potential to be concentrated by human activities, routine tissue analysis is a valuable tool in managing a watershed.

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Table 1: A comparison of NIST-certified levels of heavy metals (ppm) from mussel tissue compared to experimental values extracted by ICP-MS and our control (acid blank) shown with mean ± Students *t*-value (for a 95% confidence interval).

	As	Cd	Cu	Fe
NIST-Certified	13.3 ± 1.8	0.82 ± 0.16	4.02 ± 0.33	171 ± 4.9
Experimental ICP-MS	10 ± 0.45	0.58 ± 0.04	9.32 ± 4.3	108 ±19
Control	0.002 ± 0.002	0.001 ± 0.0008	0.36 ± 0.12	1.1 ± 1.8

	Pb	Se	Zn
NIST-Certified	1.19 ± 0.18	1.8 ± 0.15	137 ± 13
Experimental ICP-MS	1.4 ± 0.66	2.3 ± 0.38	122 ± 20
Control	0.032 ± 0.01	0.001 ± 0.004	1.01 ± 0.25

Table 2: ICPMS values of non-certified metals, mean ± students T-value for a 95% confidence interval, for acid blanks (n: 9) shown in ppm (mg/kg dry weight).

Al	Ba	Ca	Co
3.11 ± 0.49	0.052 ± 0.005	9.7 ± 2.6	0.006 ± 0.007

Cr	K	Mg	Mn
0.054 ± 0.01	2.4 ± 1.2	0.96 ± 0.36	0.032 ± 0.04

Na	Ni	Ni
61 ± 11.5	0.029 ± 0.007	0.029 ± 0.007

Table 3: Average Hydropsychidae concentrations (mg/kg dry weight) in the Rouge River Watershed and other locations with various land uses ranging from rural to urban. The (-) designates that metal was not analyzed. Rouge River land uses are based on the drainage area of the most downstream sampling point of each river branch.

River Location	Land Use	As	Cd	Cu	Cr	Mn	Ni	Pb	Zn
Ner, and Bzura River Lotz, Poland[1]	urban	-	7.5	37	5.3	-	-	75	300
Guadiamar River SW Spain[2]	strip mining	12	6	96	-	-	-	43	2337
Guadiamar River SW Spain[2]	moderate agriculture	17	1.2	23	-	-	-	36	151
Ebro River Flix, Spain[3]	industry (chemical plant discharge)	-	0.19	-	4.6	477	5.1	3	148
Ebro River Flix, Spain[3]	agriculture, light industry	-	0.06	-	4	140	3.1	3	131
Upper Branch Detroit, Michigan	residential (59%) commercial (14%) industrial (4%) rural (8%)	2.1	0.17	35.5	5.7	1473	4	8.2	161
Main Branch Detroit, Michigan	residential (68%) commercial (12%) industrial (2%) rural (6%)	3	0.19	33	4.2	1095	3.4	3.3	178
Middle Branch Detroit, Michigan	residential (36%) commercial (7%) industrial (9%) rural (20%)	2	0.21	31.4	4.8	1478	3.8	4.8	149
Lower Branch Detroit, Michigan	residential (24%) commercial (3%) industrial (4%) rural (20%)	2.1	0.21	36	4.6	1544	4.6	3.4	150
Rouge Watershed Detroit, Michigan	residential (49%) commercial (9%) industrial (5%) rural (13%)	2.4	0.2	33.2	4.7	1368	3.8	4.7	160

Data from: [1]Tszedel, 2015; [2] Sola, 2004; [3] Cid, 2010

Table 4: Average Hydropsychidae metal concentration (mg/kg dry weight) that significantly differed between the four branches of the Rouge River based on ANOVA (ppm).

	Main Rouge $\bar{X} \pm SE$	Upper Rouge $\bar{X} \pm SE$	Middle Rouge $\bar{X} \pm SE$	Lower Rouge $\bar{X} \pm SE$	$F_{(3, 178)}$	P- level
Al	425 ± 41	627 ± 77	547 ± 76	797 ± 94	6.2	0.0005
As	3.1 ± 0.44	2.1 ± 0.21	2.0 ± 0.24	2.1 ± 0.28	3.2	0.0001
Ca	9195 ± 791	6514 ± 658	6266 ± 549	5969 ± 834	4.9	0.003
Co	1.47 ± 0.15	1.53 ± 0.18	1.58 ± 0.18	2.6 ± 0.55	3.6	0.01
Fe	250 ± 18	280 ± 31	251 ± 25	366 ± 49.7	2.6	0.005
K	2209 ± 121	3066 ± 165	2713 ± 263	2511 ± 131	4.5	0.004
Mg	1544 ± 85	1708 ± 149	1365 ± 144	1556 ± 122	3.8	0.01
Mn	1096 ± 123	1474 ± 270	1478 ± 187	1544 ± 185	3.1	0.03
Na	3712 ± 300	8292 ± 3404	5903 ± 1375	6971 ± 1408	3	0.03
Ni	3.4 ± 0.18	4.0 ± 0.30	3.8 ± 0.43	4.6 ± 0.37	3.1	0.03
Se	1.8 ± 0.08	1.4 ± 0.016	1.6 ± 0.25	1.45 ± 0.10	3.9	0.01

Table 5: Average Hydropsychidae metal concentration (mg/kg dry weight) that significantly differed between sampling years in the Rouge River watershed based on ANOVA (ppm).

	2006 $\bar{X} \pm SE$	2008 $\bar{X} \pm SE$	2012 $\bar{X} \pm SE$	2015 $\bar{X} \pm SE$	$F_{(3, 178)}$	P-level
Al	471 ± 61	529 ± 58	382 ± 35	743 ± 93	6.6	0.0003
As	1.9 ± 0.19	3.2 ± 0.52	1.6. ± 0.11	2.7 ± 0.34	7.3	0.0001
Ba	29 ± 3.5	60 ± 10.3	42.1 ± 5.7	58 ± 5.7	8.7	4.5E-06
Cd	0.29 ± 0.043	0.21 ± 0.015	0.15 ± 0.014	0.18 ± 0.019	6	0.0006
Co	1.16 ± 0.09	1.60 ± 0.11	1.15 ± 0.089	2.37 ± 0.31	9.2	1.1E-05
Fe	2180 ± 245	3140 ± 257	1851 ± 129	3303 ± 324	10.3	0.005
K	2425± 185	2531 ± 155	2227 ± 105	2989 ± 304	2.7	0.04
Mn	902 ± 118	1124 ± 95	926 ± 76	2122 ± 246	17.2	7.7E-10
Na	6619 ± 2943	3968 ± 332	2920 ± 197	8543 ± 1669	16.2	2.4E-09
Pb	3.9 ± 0.48	7.5 ± 2.9	4.1 + 1.2	3.7 ± 0.71	5	0.002
Sr	19.9 ± 2.1	26.6 ± 2.4	32.2 ±3.7	30.6 ± 2.9	5.6	0.001

Table 6: Average Hydropsychidae metal concentration (mg/kg dry weight) that significantly differed between sampling years at sites within the Main Branch of the Rouge River based on ANOVA (ppm).

	2006 $\bar{X} \pm SE$	2008 $\bar{X} \pm SE$	2012 $\bar{X} \pm SE$	2015 $\bar{X} \pm SE$	$F_{(3, 52)}$	P-level
As	2.2 ± 0.35	5.4 ± 1.4	1.8 ± 0.18	3.0 ± 0.95	6.3	0.001
Ba	27.1 ± 10.8	53.5 ± 10.3	41.3 ± 7.1	43.1 ± 5.1	2.8	0.008
Cd	0.33 ± 0.10	0.18 ± 0.016	0.16 ± 0.025	0.16 ± 0.019	3.3	0.028
Cr	3.23 ± 0.55	5.24 ± 0.49	4.35 ± 0.32	3.47 ± 0.47	4.5	0.007
Cu	35.0 ± 2.5	37.6 ± 3.3	31.5. ± 1.8	28.3 ± 2.1	2.9	0.041
Fe	2064 ± 422	3589 ± 390	2002 ± 236	2402 ± 327	4.8	0.005
K	1785 ± 433	2488 ± 261	2042 ± 136	2513 ± 179	3.1	0.035
Mn	418 ± 89.8	1231 ± 215	934 ± 147	1773 ± 376	9.7	0.00004
Na	2802 ± 486	4786 ± 805	2713 ± 189	4886 ± 697	7.1	0.0004
Ni	3.8 ± 0.39	4.3 ± 0.45	2.9 ± 0.21	2.5 ± 0.15	6.4	0.0009
Pb	3.5 ± 0.65	4.8 ± 0.46	2.6 + 0.32	2.6 ± 0.20	8.3	0.0001

Table 7: Pearson correlation values (r) for metal accumulation and upstream drainage areas with different land uses. Strength of correlation coefficients are as follows: $0.1 < | r | < 0.3$ weak correlation, $0.3 < | r | < 0.5$ moderate correlation, $| r | > 0.5$ strong correlation.

Land Use	Al	As	Ba	Cd	Co	Cr	Cu
Commercial or Industrial	-0.001	-0.056	-0.050	-0.199	-0.031	0.045	-0.020
Residential	-0.049	0.230	-0.091	-0.025	0.009	0.111	0.121
Undeveloped	-0.008	-0.208	0.064	0.039	-0.051	-0.109	-0.138
Agricultural	0.118	-0.180	0.206	0.100	0.154	-0.117	-0.053

Land Use	Fe	Mn	Ni	Pb	Se	Sr	Zn
Commercial or Industrial	-0.087	0.020	-0.124	0.069	0.221	0.167	0.035
Residential	-0.073	-0.137	-0.004	0.012	0.315	0.116	0.187
Undeveloped	0.096	0.144	-0.001	-0.033	-0.367	-0.143	-0.204
Agricultural	0.060	0.160	0.102	-0.056	-0.165	-0.072	-0.106

Table 8: Average Hydropsychidae Metal accumulation (ppm) that significantly differed in drainage areas with different land uses based on ANOVA. Each drainage area contained a number of upstream Field IDs. Land uses are based on the drainage area of the most downstream Field ID of the sub-watersheds.

Field ID	Commercial or Industrial	Residential	Agriculture	Undeveloped
Up1, Up2, Min2, Min3	5%	55%	0.07%	28%
Fel1, Fel4, Fel5, Fel6	1.5%	37%	15%	36%
Main1, Main3, Main 4, Main4.5, Main 5, Main 11,	3.5%	44%	0.05%	46%
Wall0, Wall1, Wall2, Wall3	8.6%	50%	0%	48%
Ton0.5, Ton1	1.9%	50%	0%	48%
Peb1, Peb2, Peb3	9%	58%	0%	31%

Field ID	Al ($\bar{X} \pm$ SE)	As ($\bar{X} \pm$ SE)	Cd ($\bar{X} \pm$ SE)	Co ($\bar{X} \pm$ SE)
Up1, Up2, Min2, Min3	546 $\pm$ 64	2.0 $\pm$ 0.2	0.2 $\pm$ 0.02	1.4 $\pm$ 0.2
Fel1, Fel4, Fel5, Fel6	949 $\pm$ 174	2.0 $\pm$ 0.2	0.3 $\pm$ 0.03	1.7 $\pm$ 0.2
Main1, Main3, Main 4, Main4.5, Main 5, Main 11,	357 $\pm$ 55	4.4 $\pm$ 0.9	0.1 $\pm$ 0.01	1.5 $\pm$ 0.3
Wall0, Wall1, Wall2, Wall3	524$\pm$ 90	1.6 $\pm$ 0.2	0.25 $\pm$ 0.05	1.2 $\pm$ 0.11
Ton1/2, Ton1	448 $\pm$ 56	2.9 $\pm$ 0.6	0.25 $\pm$ 0.05	2.5 $\pm$ 0.6
Peb1, Peb2, Peb3	310 $\pm$38	1.7 $\pm$ 0.1	0.1 $\pm$ 0.02	1.3 $\pm$ 0.2
$F_{(5,94)}$	5.3	5	2.6	2.5
P-level	0.0003	0.0004	0.03	0.04

Field ID	Cr ($\bar{X} \pm$ SE)	Mn ($\bar{X} \pm$ SE)	Pb ($\bar{X} \pm$ SE)	Zn ($\bar{X} \pm$ SE)
Up1, Up2, Min2, Min3	5.6 $\pm$ 0.6	1546 $\pm$ 324	9.5 $\pm$ 5.1	161 $\pm$ 97
Fel1, Fel4, Fel5, Fel6	5.3 $\pm$ 0.7	1582 $\pm$ 184	2.9 $\pm$ 0.4	138 $\pm$ 14
Main1, Main3, Main 4, Main4.5, Main 5, Main 11,	3.5 $\pm$ 0.3	991 $\pm$ 231	3.4 $\pm$ 0.4	180 $\pm$ 14
Wall0, Wall1, Wall2, Wall3	4.9 $\pm$ 0.8	1117 $\pm$ 118	3.7 $\pm$ 0.5	139 $\pm$ 6
Ton0.5, Ton1	4.1 $\pm$ 0.6	2382 $\pm$ 847	2.1 $\pm$ 0.3	134 $\pm$ 12
Peb1, Peb2, Peb3	4.4 $\pm$ 0.4	1392 $\pm$ 190	2.7 $\pm$ 0.3	134 $\pm$ 9
$F_{(5,94)}$	2.8	3.2	2.6	0.02
P-level	0.019	0.01	0.03	2.9

Table 9: Pearson correlation values (r) for metal accumulation and biodiversity (stream quality score) and richness (number of taxa). Strength of correlation coefficients are as follows: 0.1 < | r | < 0.3 weak correlation, 0.3 < | r | < 0.5 moderate correlation, | r | > 0.5 strong correlation.

	Al	As	Ba	Cd	Co	Cr	Cu
Stream Quality Score (SQI)	-0.089	-0.057	0.128	0.100	0.096	-0.140	-0.241
Number of Taxa	-0.093	0.016	0.133	0.126	0.063	-0.106	-0.230

	Fe	Mn	Ni	Pb	Se	Sr	Zn
Stream Quality Score (SQI)	0.018	0.147	-0.096	-0.166	0.040	-0.071	-0.132
Number of Taxa	0.054	0.130	-0.108	-0.093	0.070	-0.064	-0.137

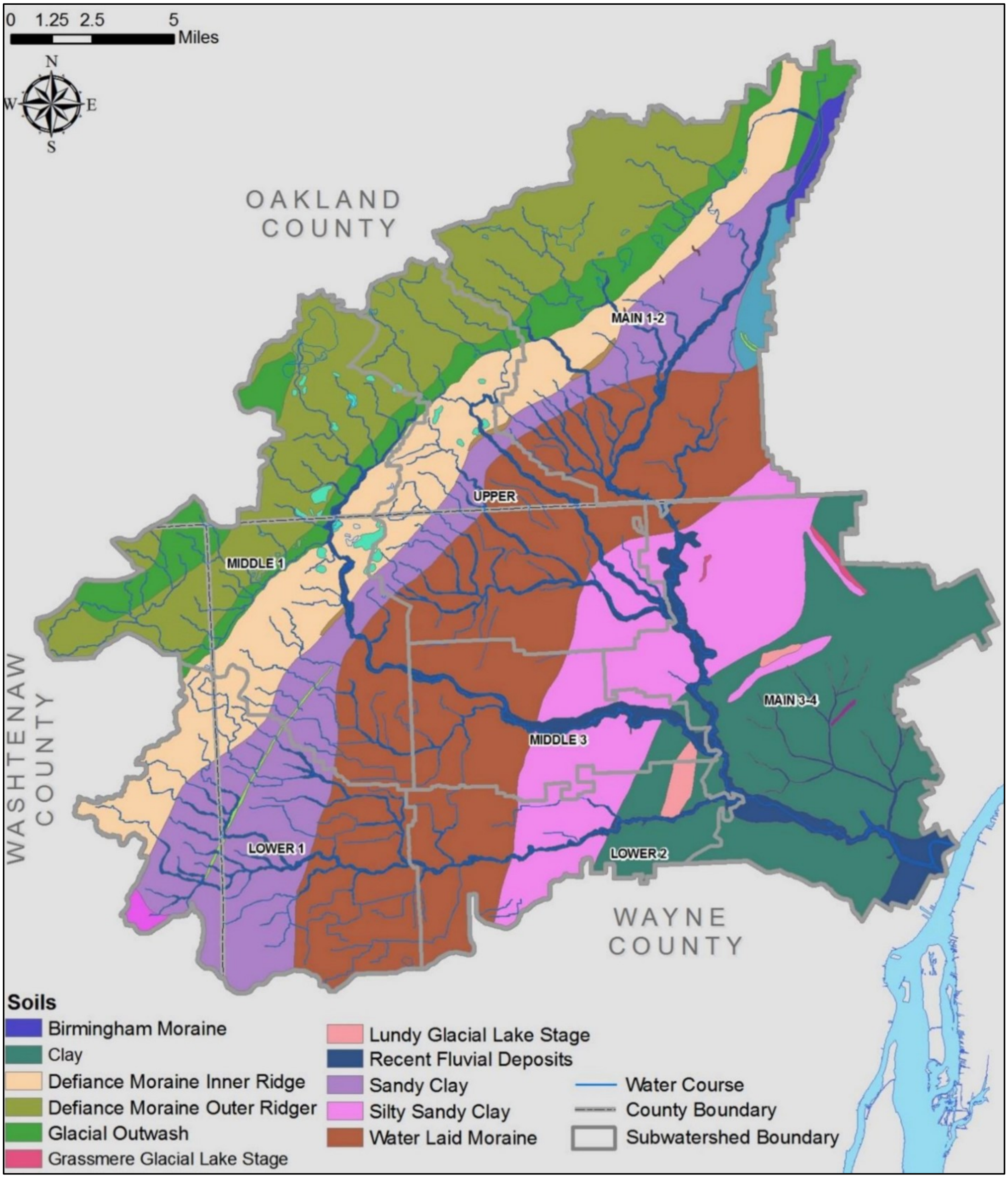


Figure 1: A map of the soil distribution within the Rouge River Watershed. Data taken from the Rouge River Watershed Management Plan, 2012.

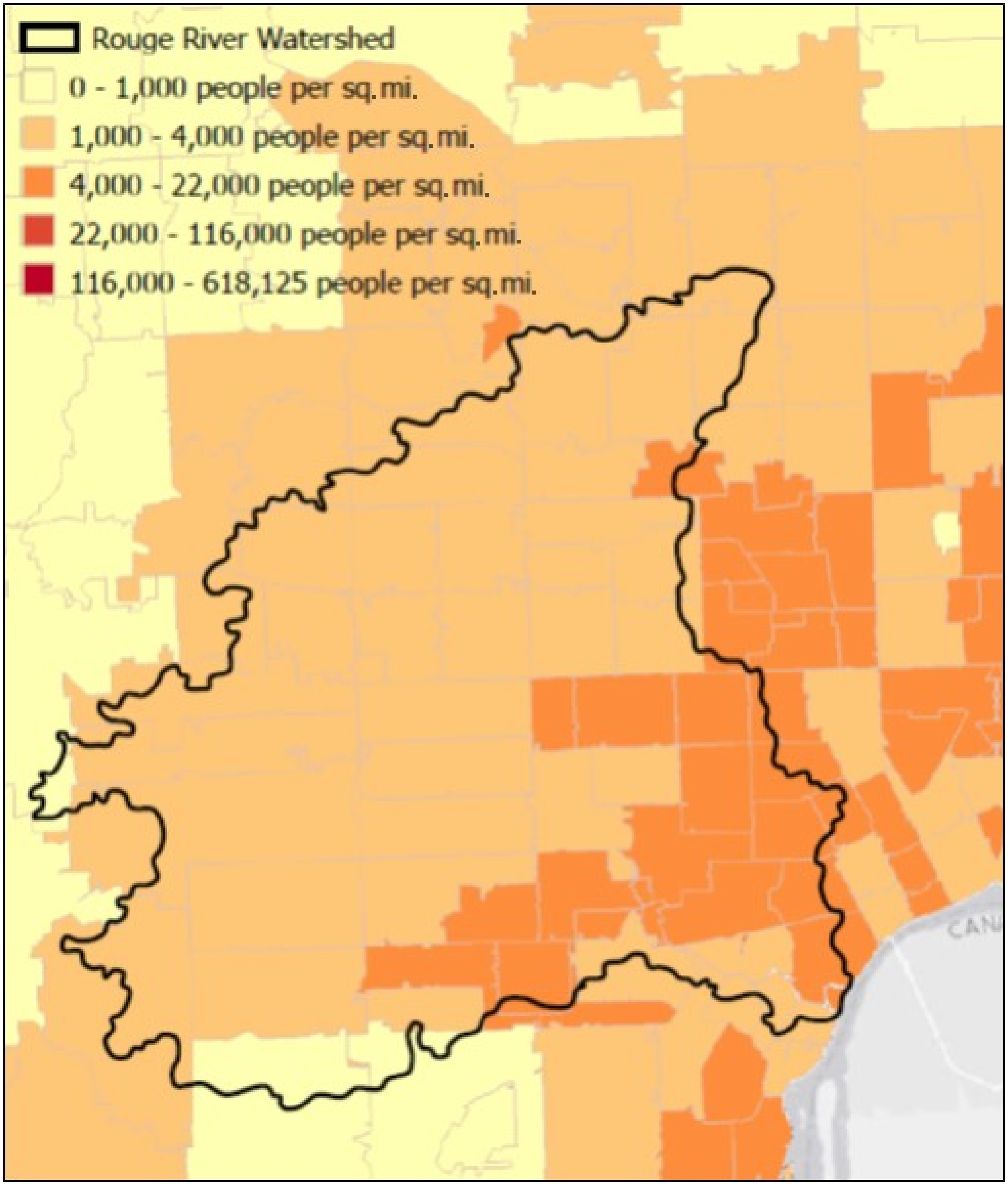


Figure 2: Population density of Rouge River watershed in 2017, data was estimated from the 2010 United States demographic census.

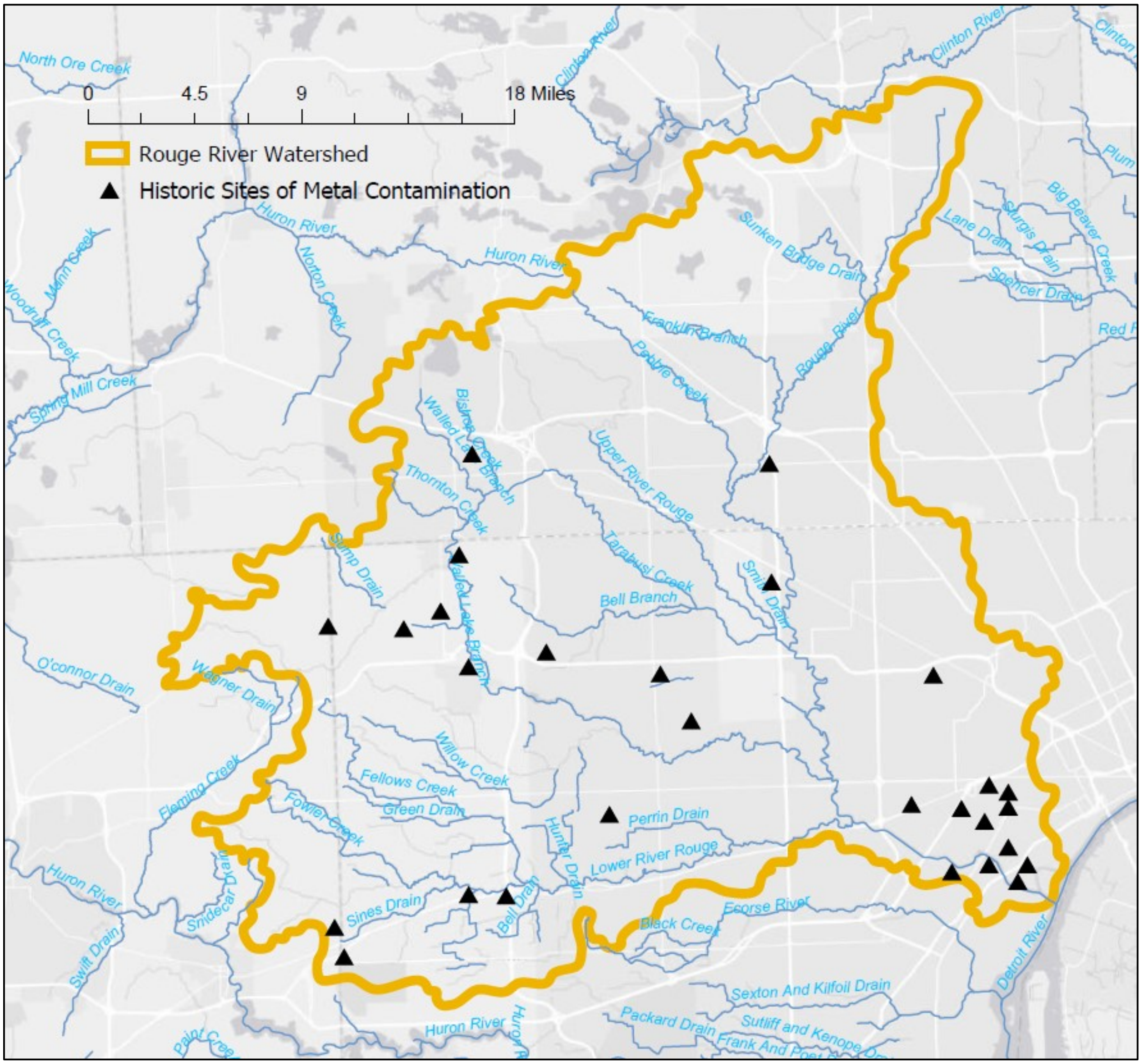


Figure 3: Known sites of point-source heavy metal (Pb, Cu, Ni, Zn, Cr, Cd, and As) pollution identified by the Michigan Environmental Response Act in 1994. Data taken from the Michigan Department of Natural Resources Rouge River Assessment, 1998.

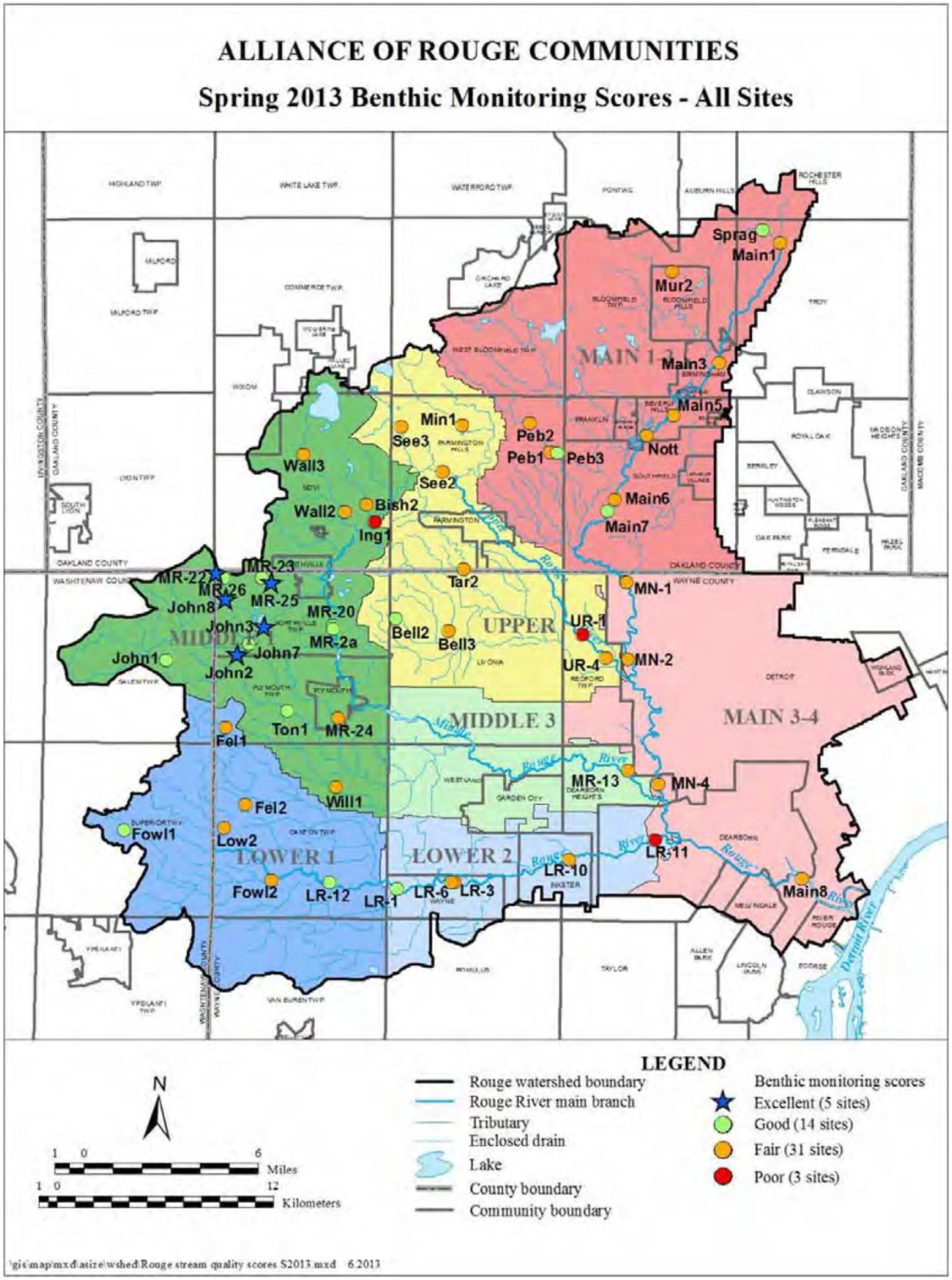


Figure 4: Benthic monitoring scores of macroinvertebrate biodiversity of the Rouge River. Figure taken from Wayne, 2013.

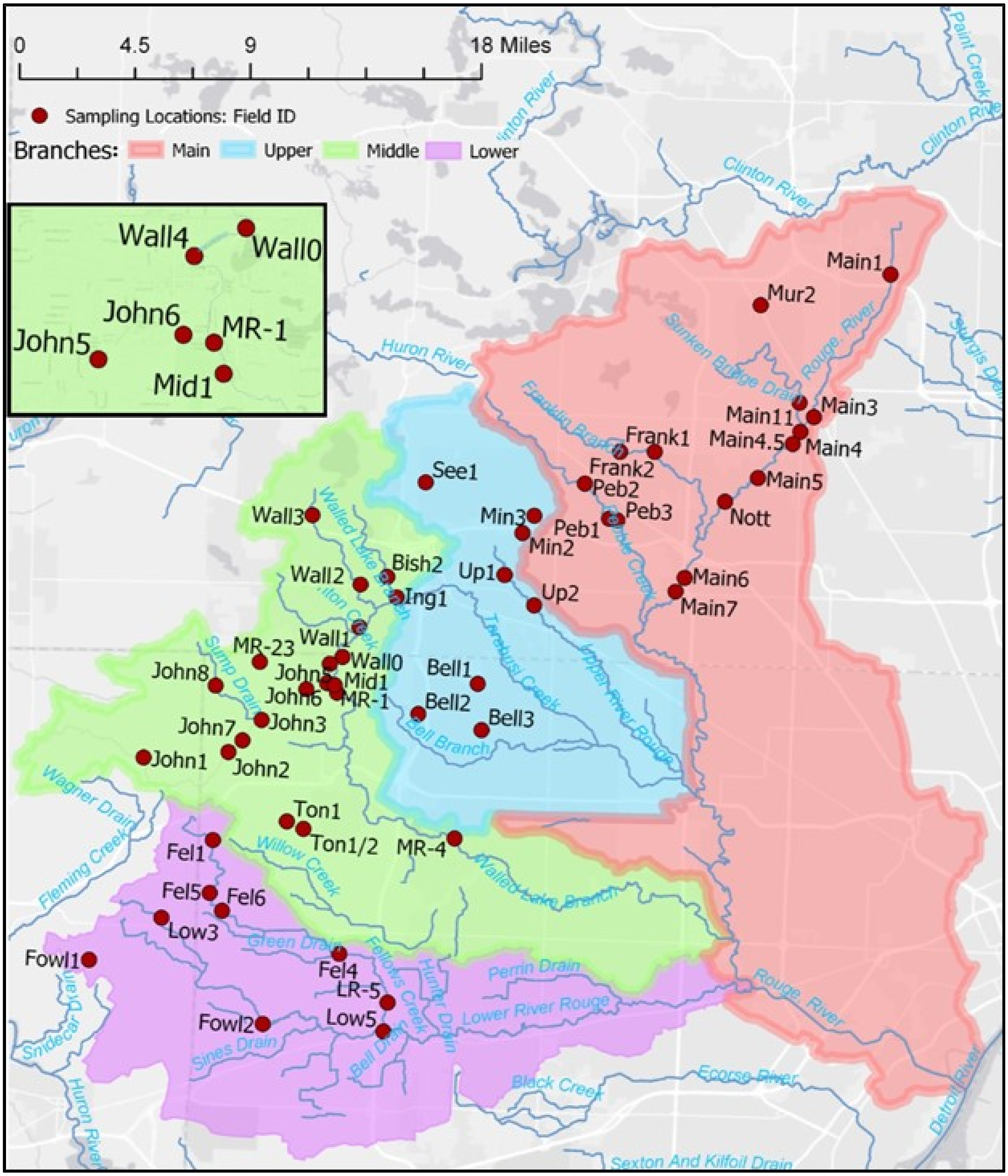


Figure 5: Map of the Hydropsychidae sampling locations labelled with their field ID throughout the Rouge River Watershed.

Figure 6: A net-spinning caddisfly of the Hydropsychidae family whose larvae develop in the benthic regions of streams and rivers (scale: 1 division = 0.1 mm). The three sclerotized dorsal plates (arrows) are characteristic of the family.

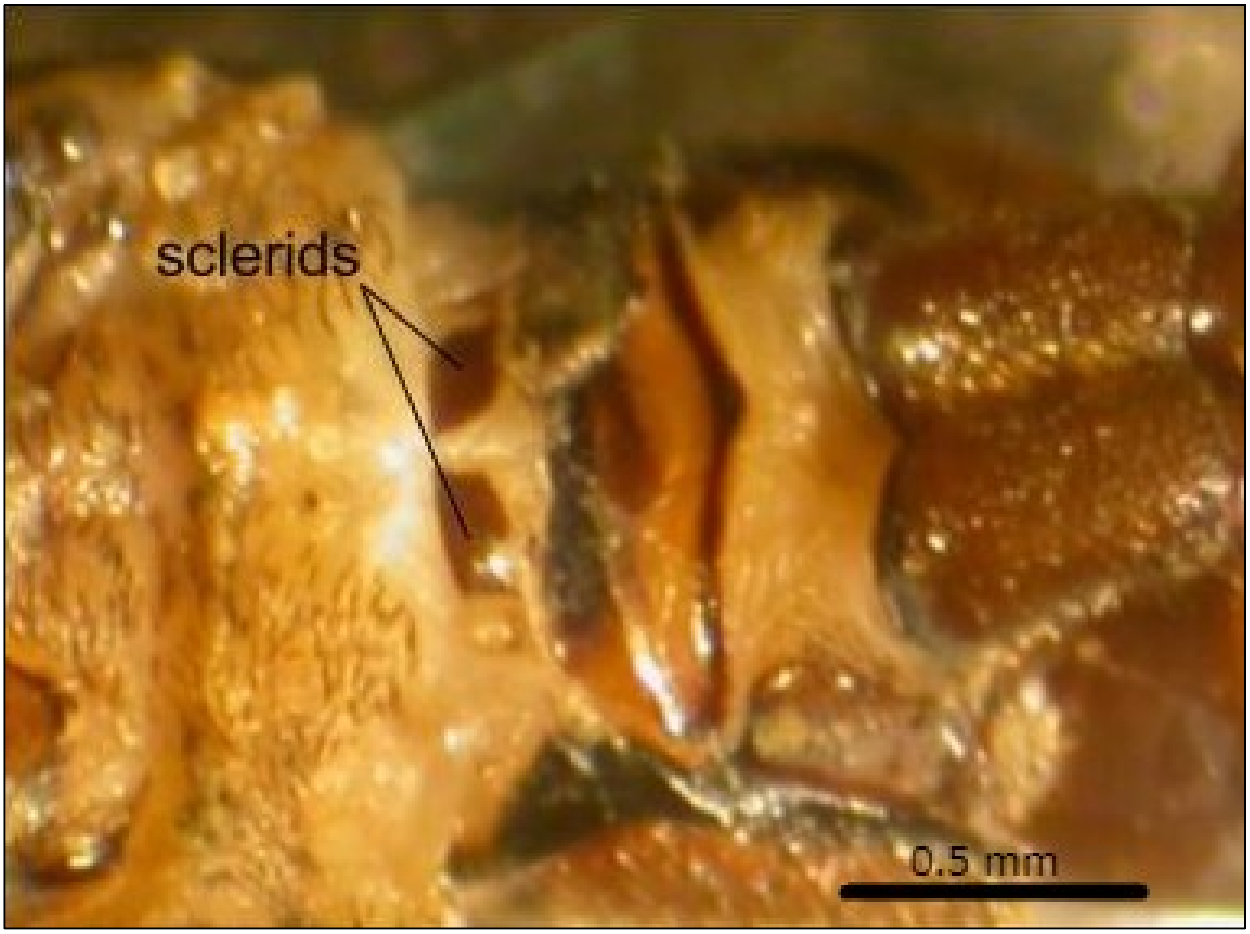


Figure 7: The *Ceratopsyche* and *Hydropsyche* genera of net-spinning caddisflies have two large sclerids (arrows) on the ventral side of the thorax, which is characteristic of these two genera.

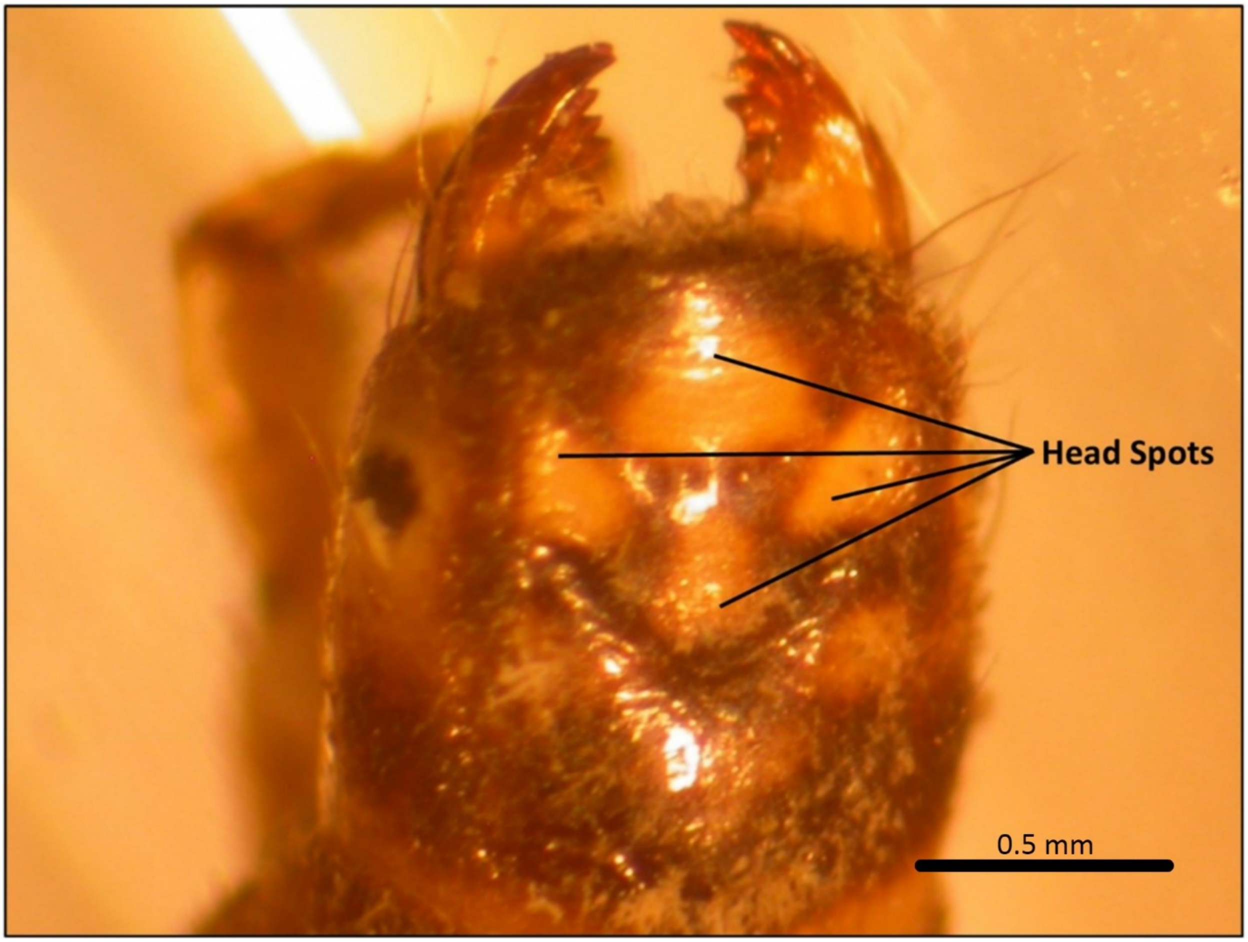


Figure 8: The *Ceratopsyche* genus has distinct spots on the dorsal surface of the head (arrows).

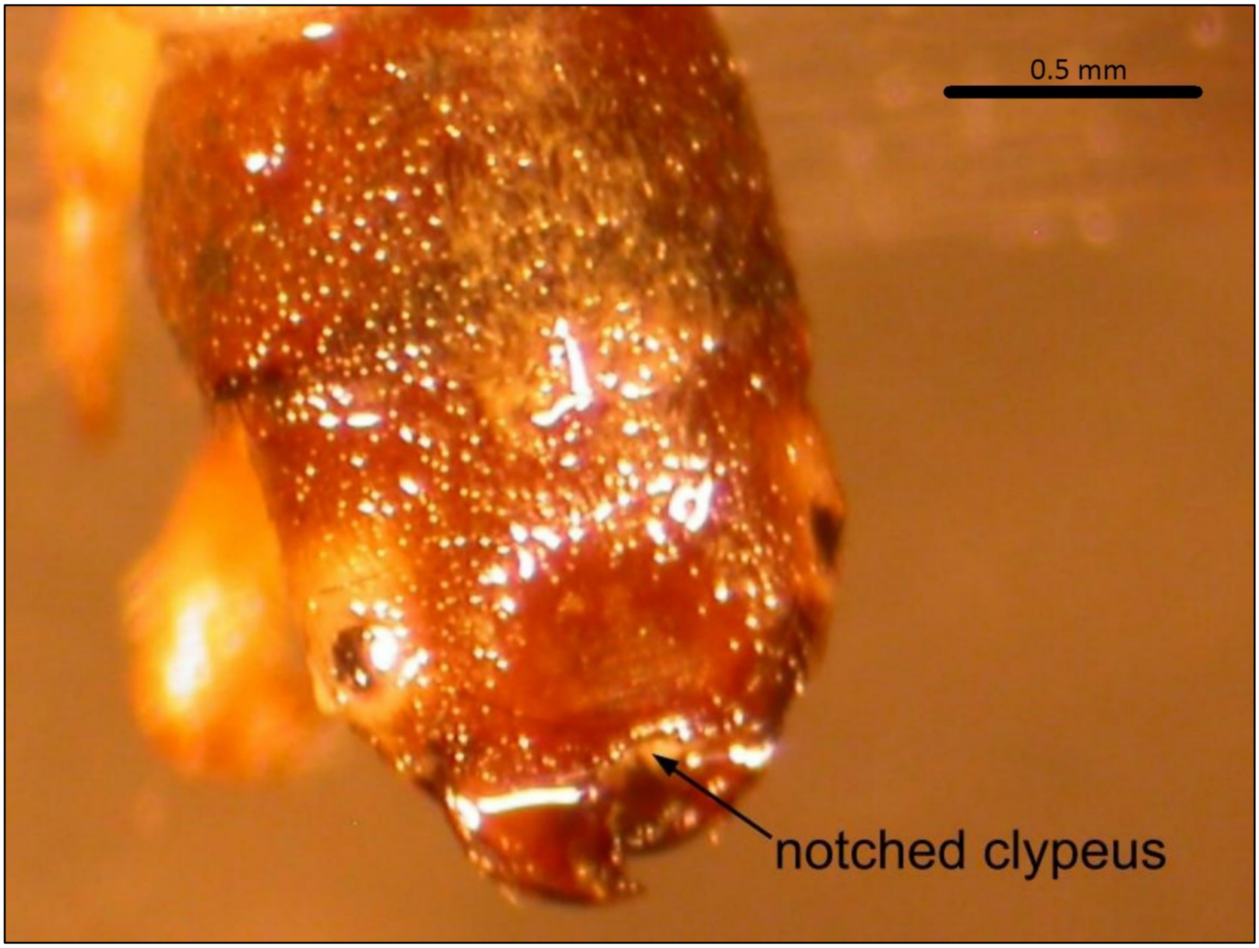


Figure 9: *Cheumatopsyche* have a characteristic notch on the anterior edge of the clypeus.

Figure 10: A Hydropsychidae larvae (scale: 1 division = 0.1 mm) with protruded anal papillae (arrow), a morphological marker correlated to certain freshwater pollutants.

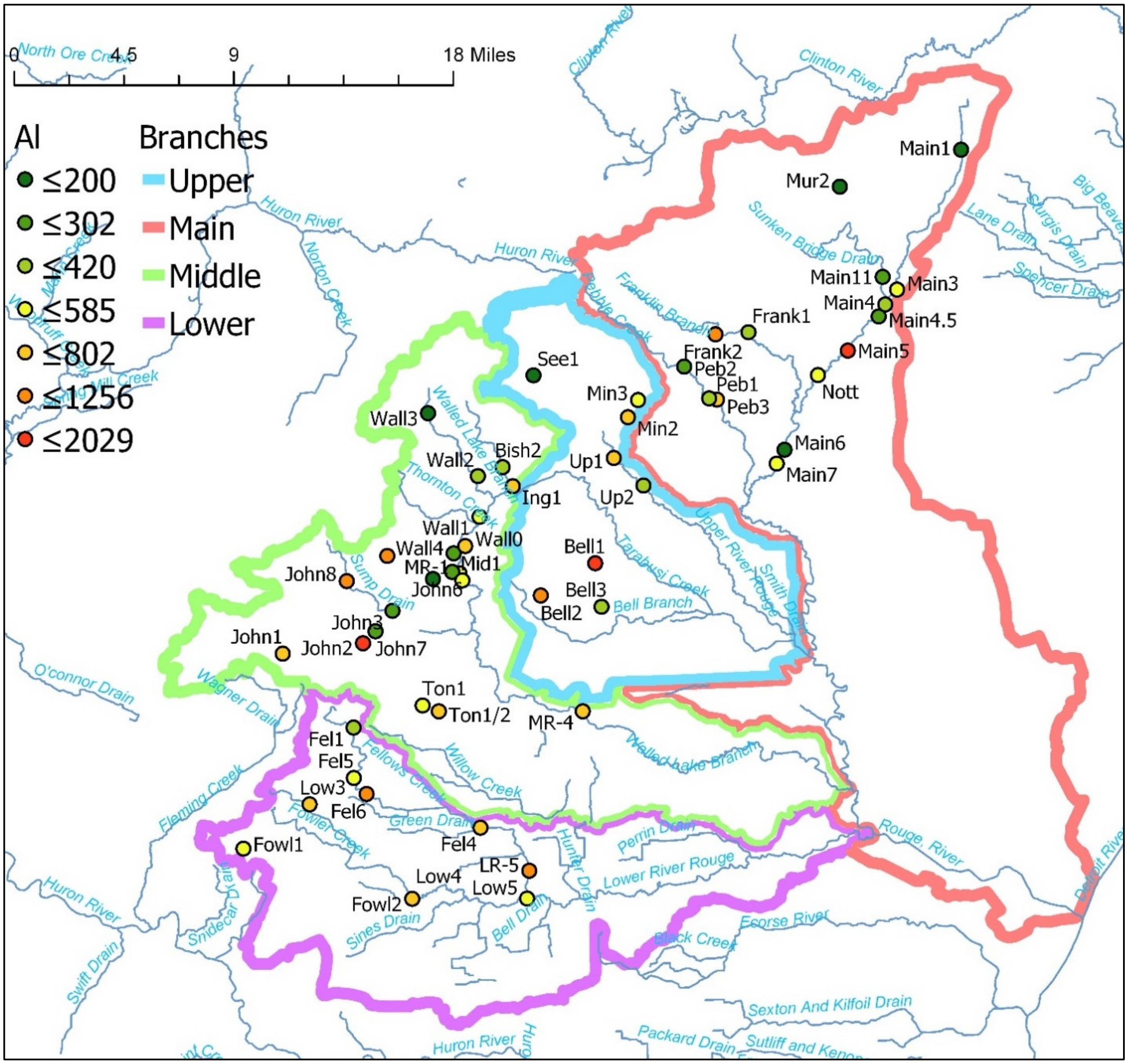


Figure 11: Maximum aluminum concentration (mg/kg dry weight) observed of Hydropsychidae at each sampling location within the four branches of the Rouge River.

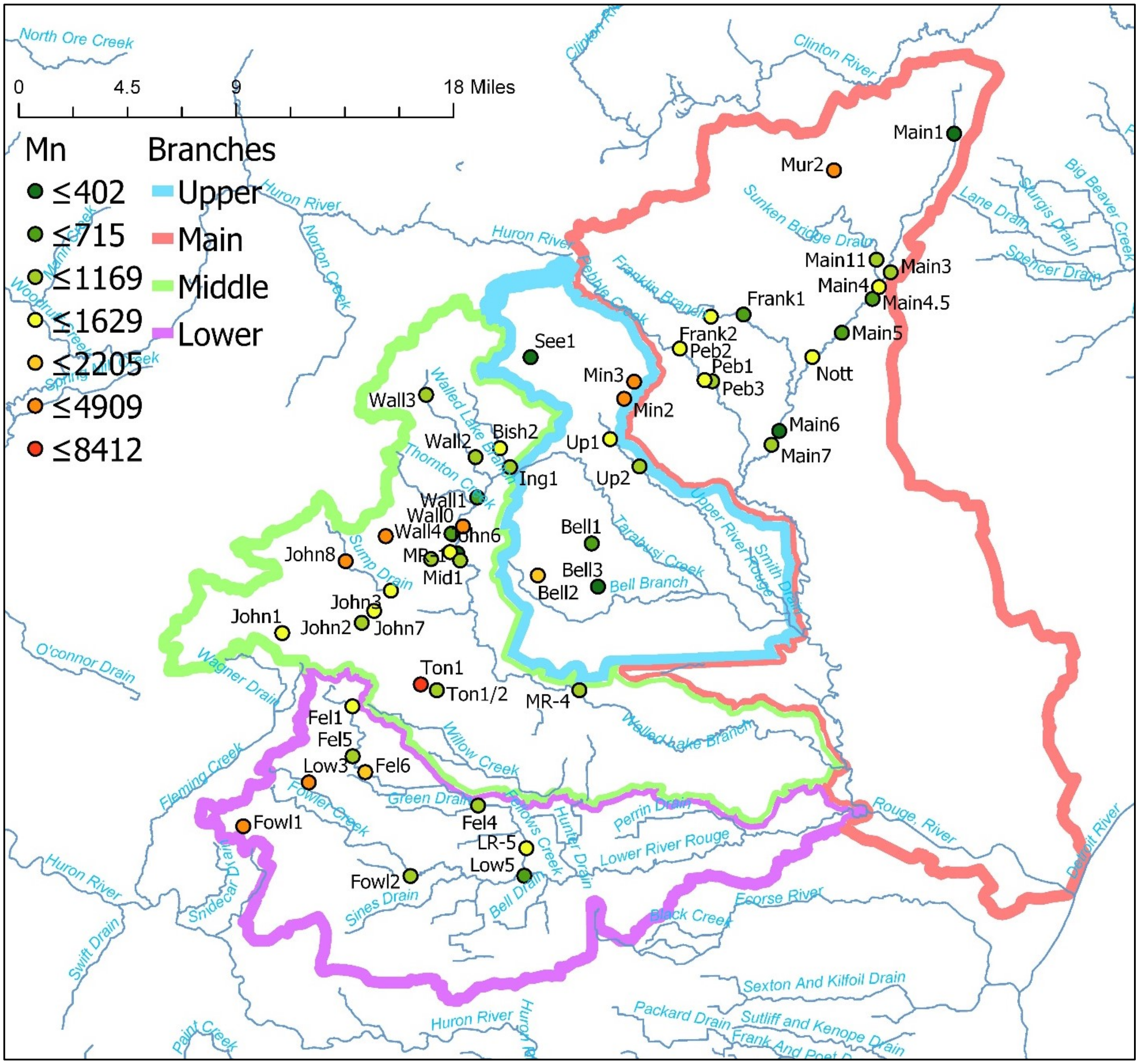


Figure 12: Maximum manganese concentration (mg/kg dry weight) observed of Hydropsychidae at each sampling location within the four branches of the Rouge River.

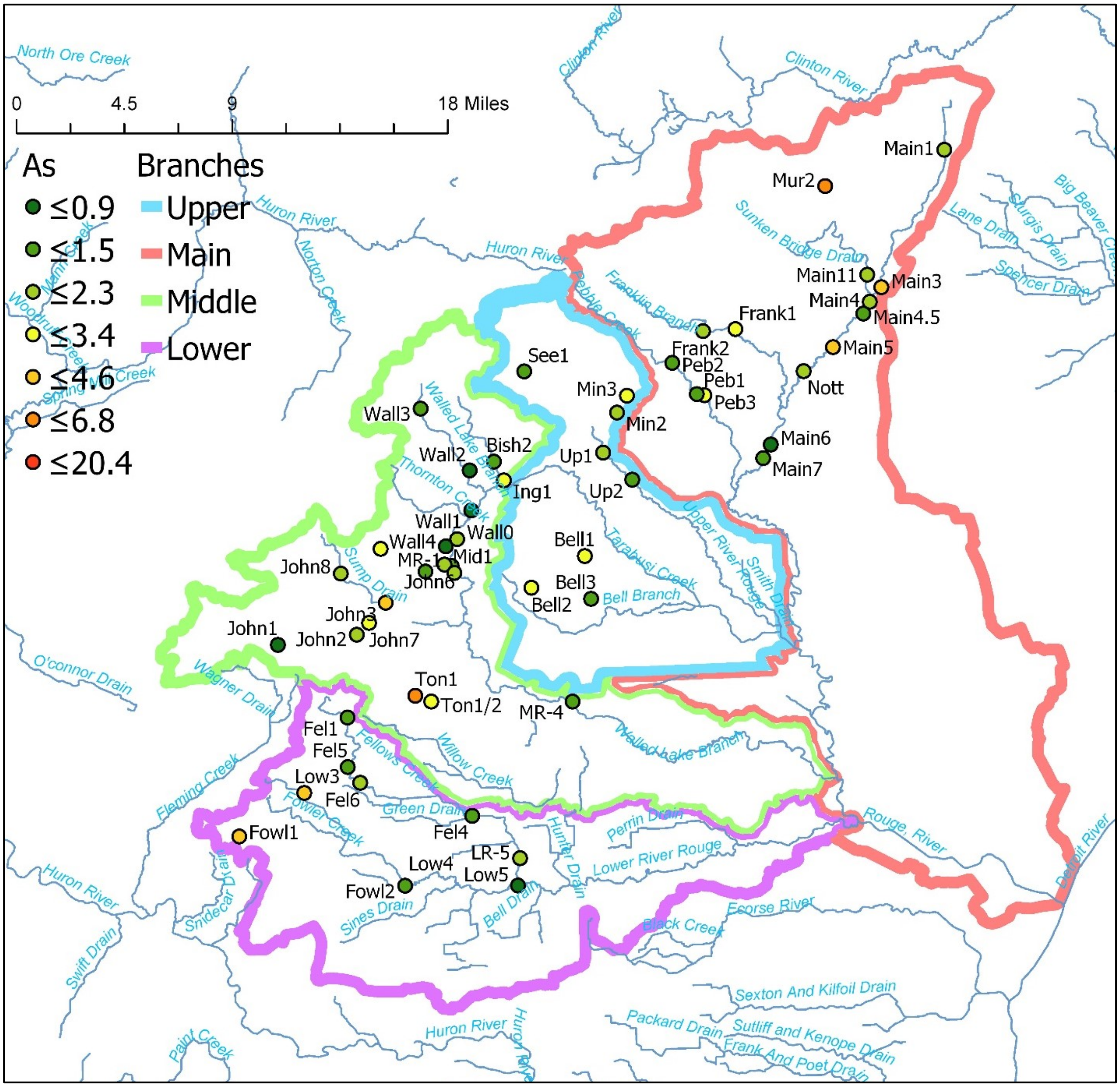


Figure 13: Maximum arsenic concentration (mg/kg dry weight) observed of Hydropsychidae at each sampling location within the four branches of the Rouge River.

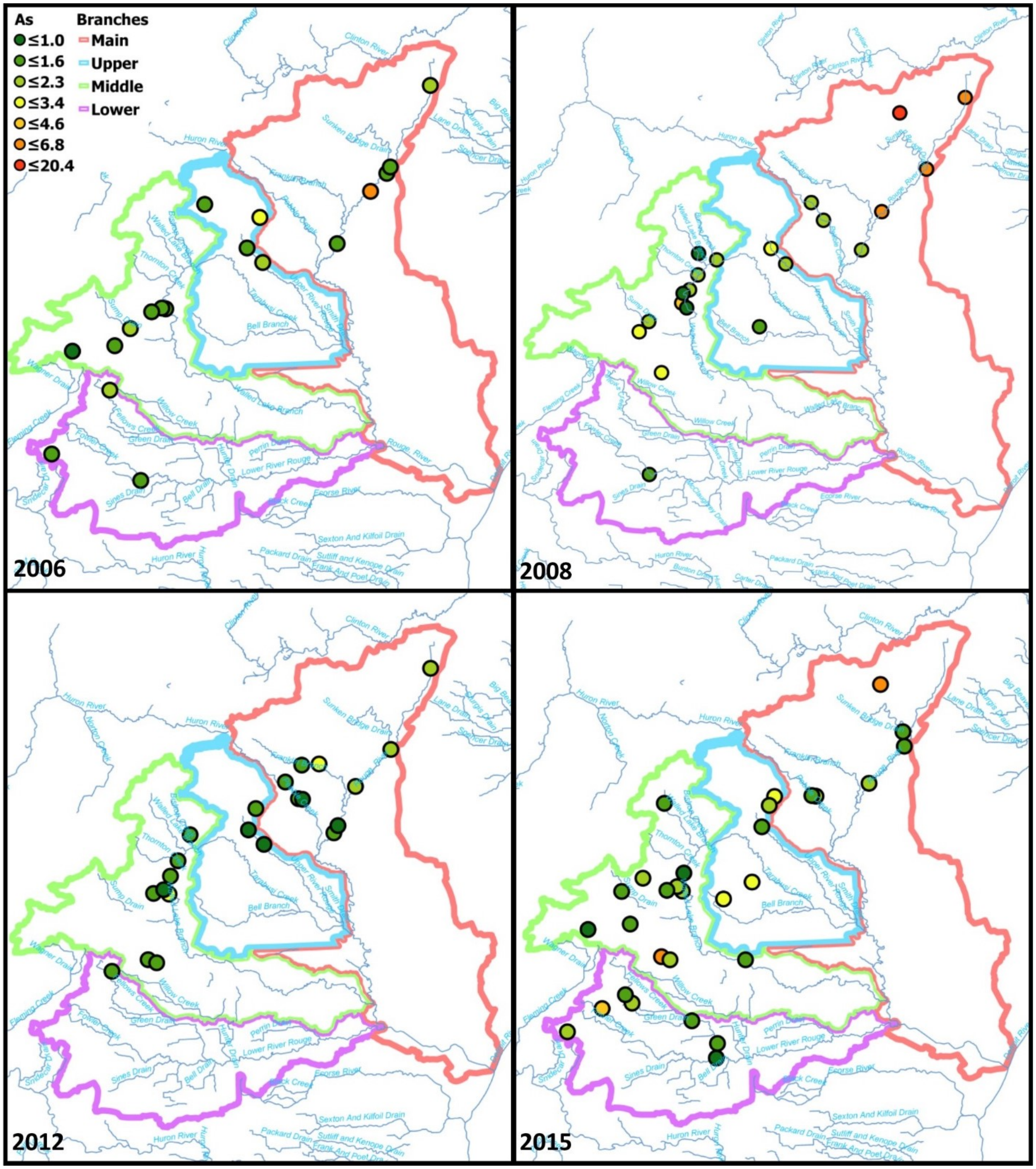


Figure 14: Maximum arsenic Hydropsychidae metal concentration (mg/kg dry weight) observed at individual sampling location for each of the four years.

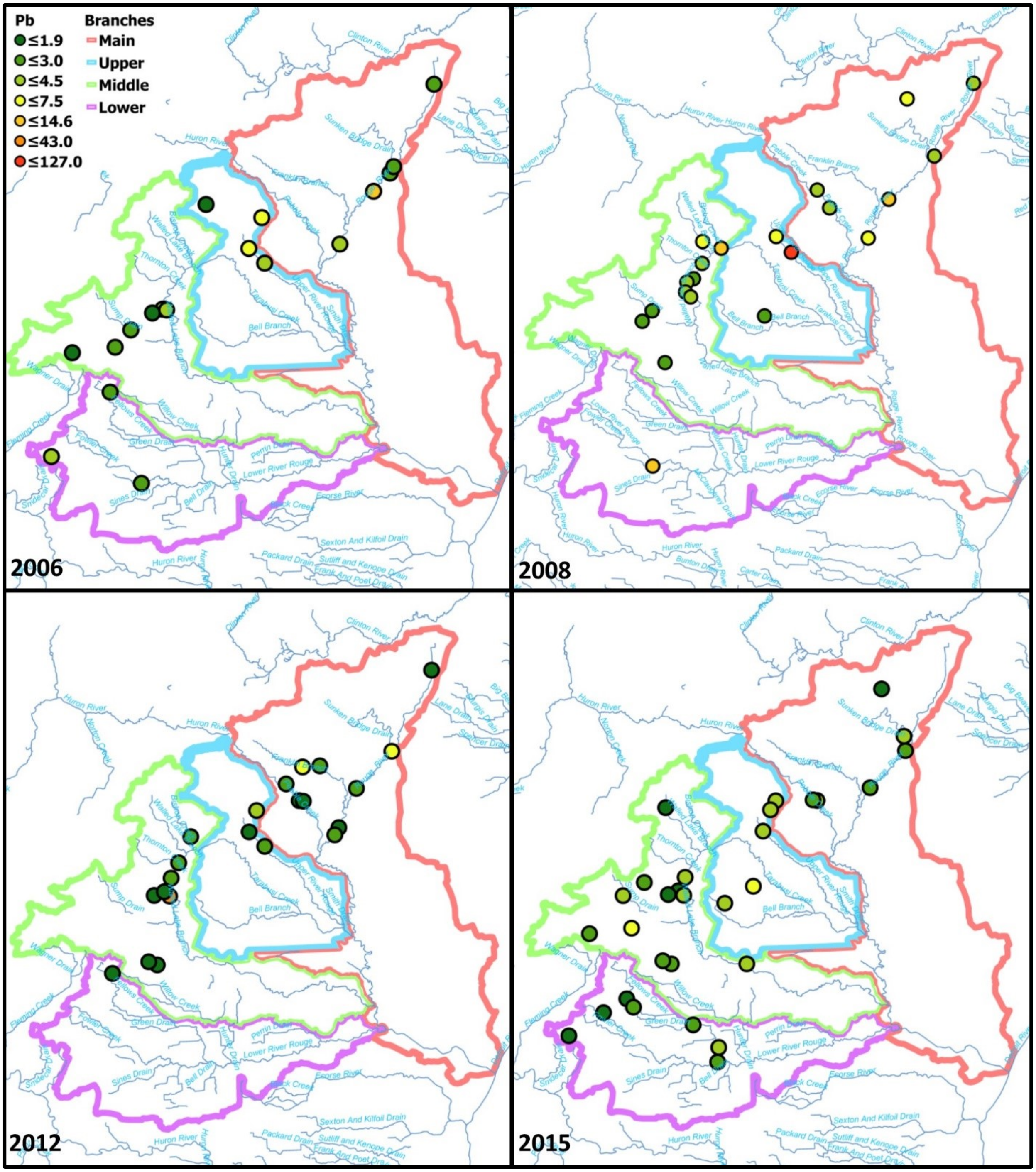


Figure 15: Maximum lead concentration (mg/kg dry weight) of Hydropsychidae at individual sampling location for each of the four years.

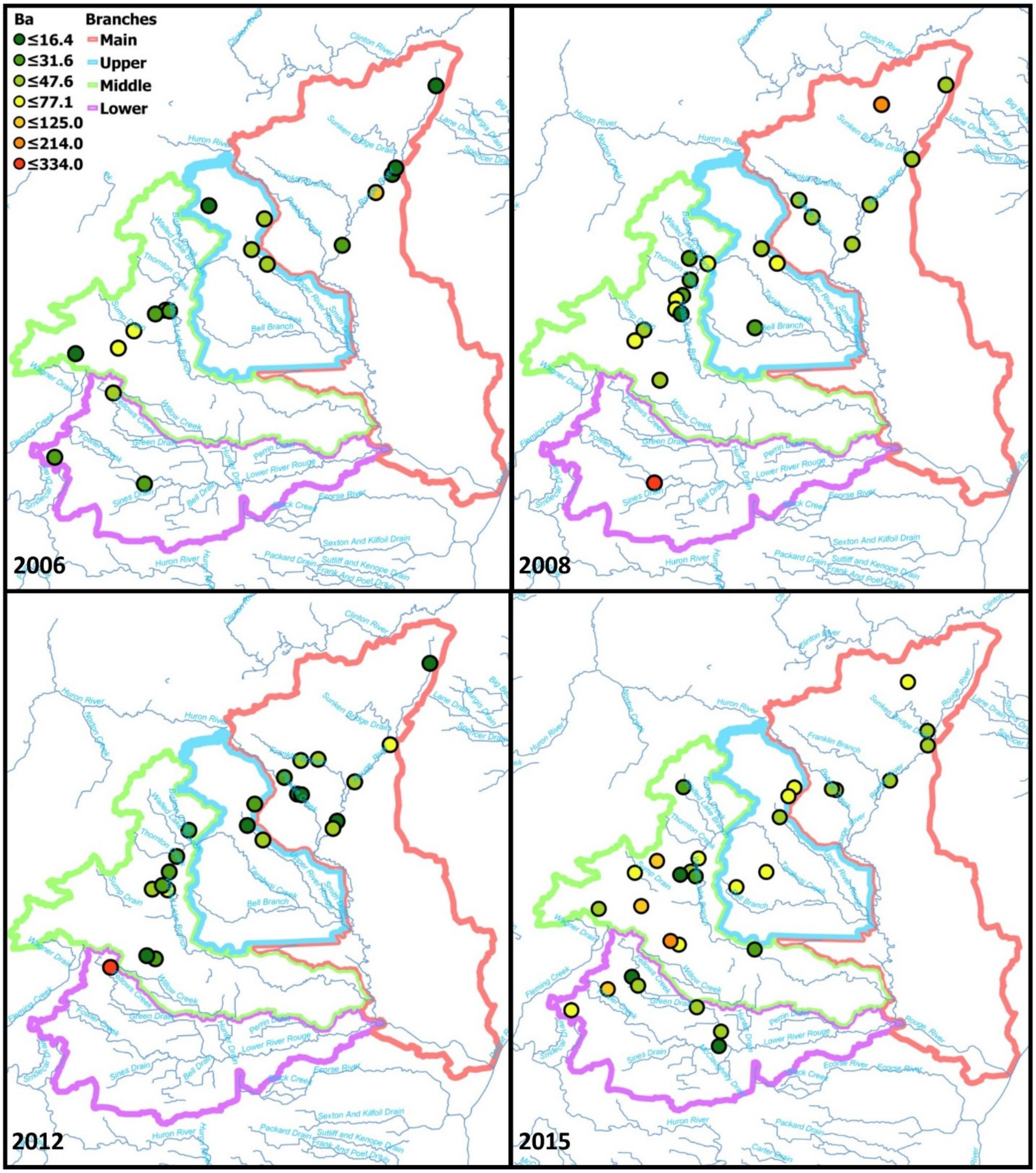


Figure 16: Maximum barium concentration (mg/kg dry weight) observed of Hydropsychidae at individual sampling location for each of the four years.

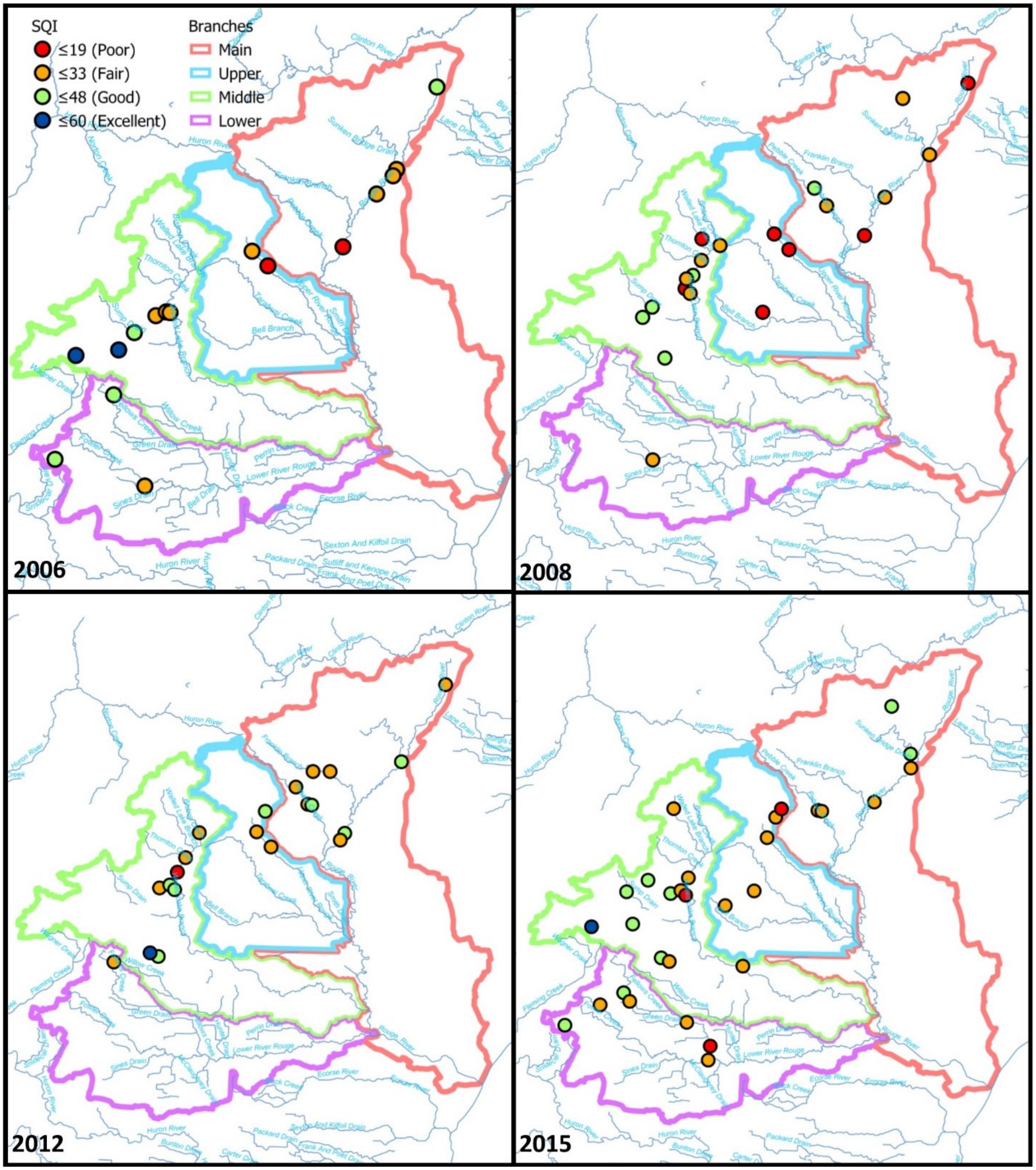


Figure 17: Stream Quality Scores (SQI) at the locations of the Hydropsychidae sampling for each of the four years. The SQI is a measure of stream health based on the biodiversity of benthic invertebrates present ranging from pollutant sensitive, somewhat sensitive and tolerant. Appendix 3 contains for the procedure used to calculate the SQI. Data was provided by Friends of the Rouge.

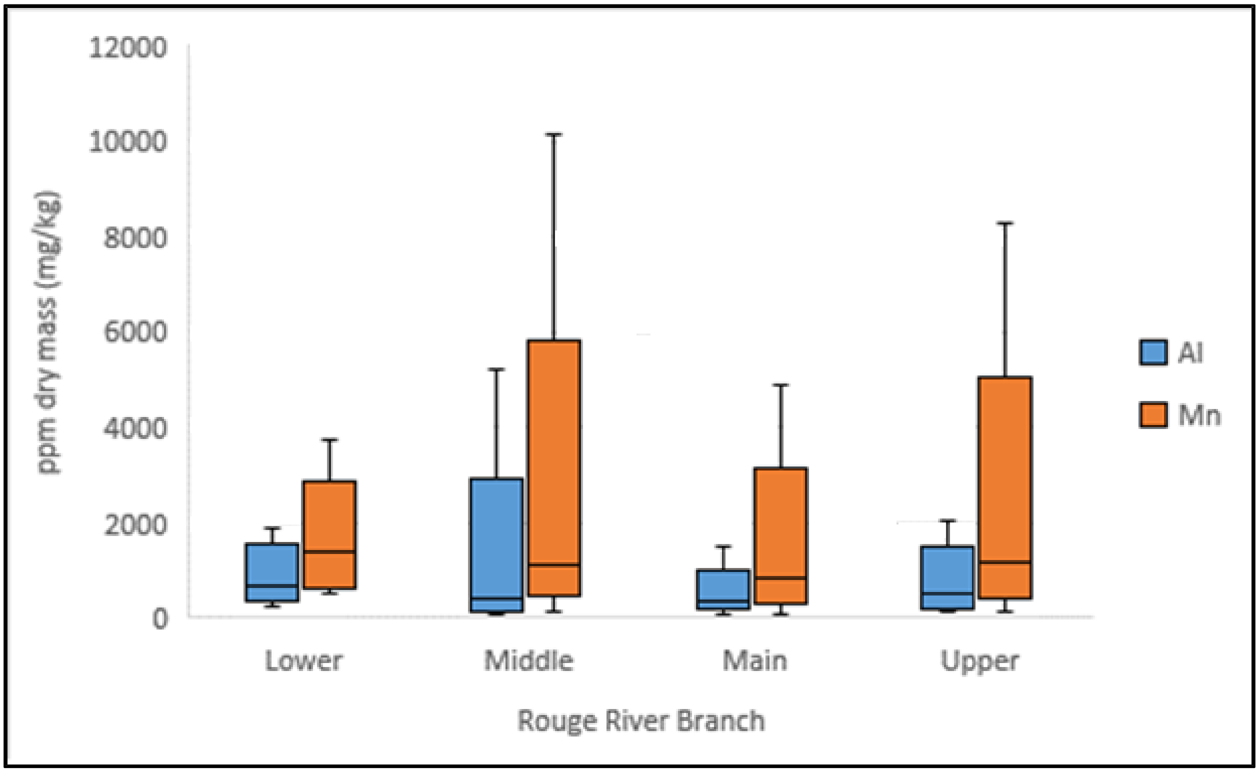


Figure 18: Hydropsychidae accumulation of aluminum and manganese from different branches of the Rouge River (top error bar: max, top of box: quartile 3, line in box: median, bottom of box: quartile 1, lower error bar: minimum).

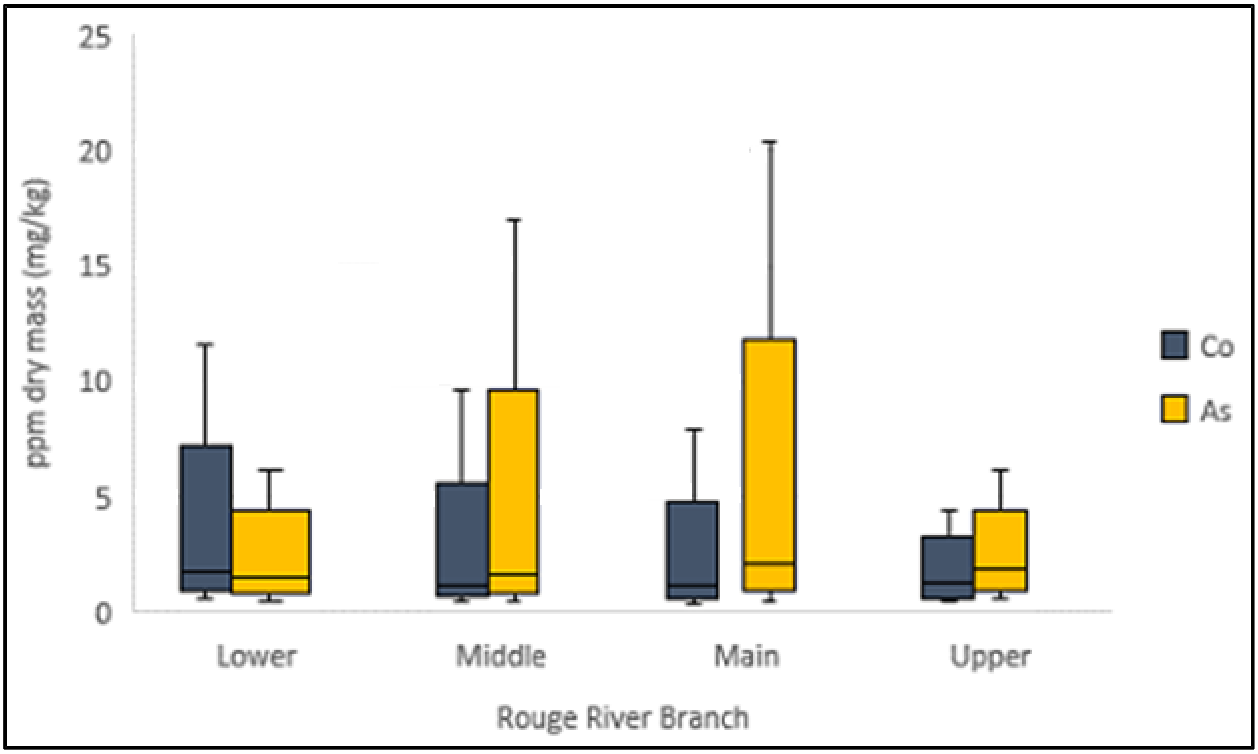


Figure 19: Hydropsychidae accumulation of cobalt and arsenic from different branches of the Rouge River (top error bar: max, top of box: quartile 3, line in box: median, bottom of box: quartile 1, lower error bar: minimum).

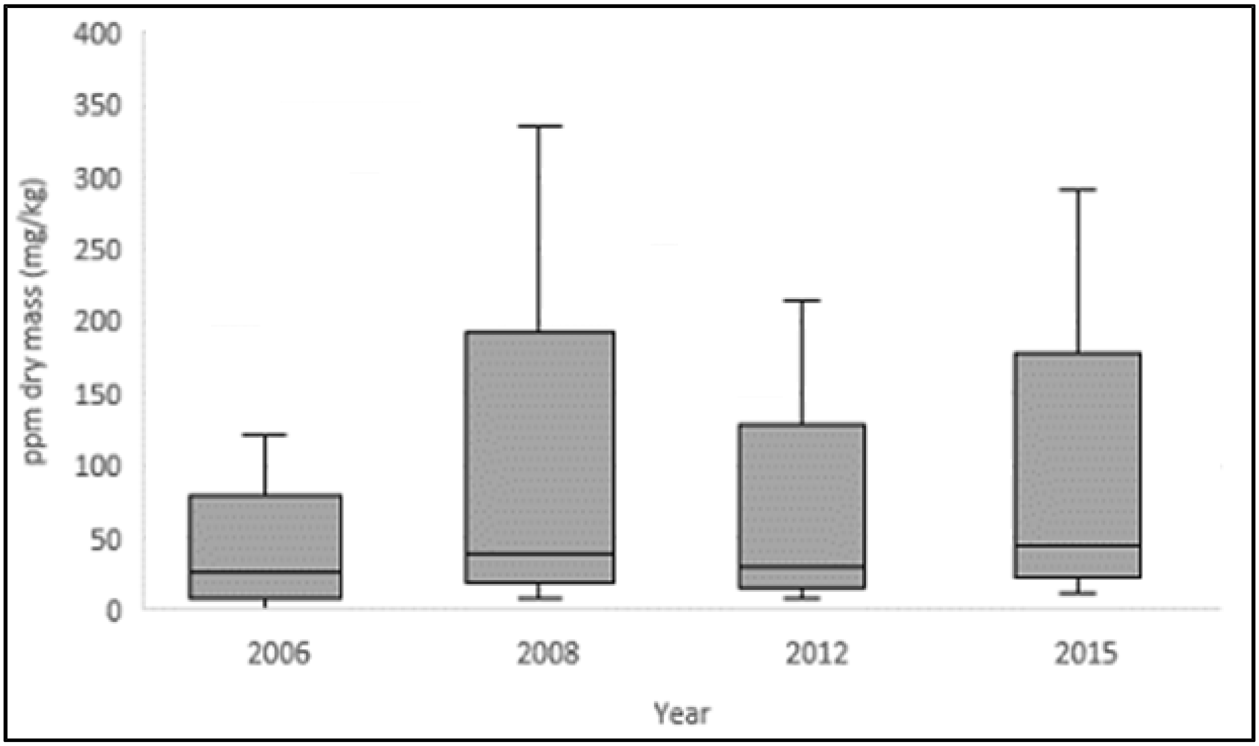


Figure 20: Hydropsychidae accumulation of barium from different sampling years in the Rouge River (top error bar: max, top of box: quartile 3, line in box: median, bottom of box: quartile 1, lower error bar: minimum).

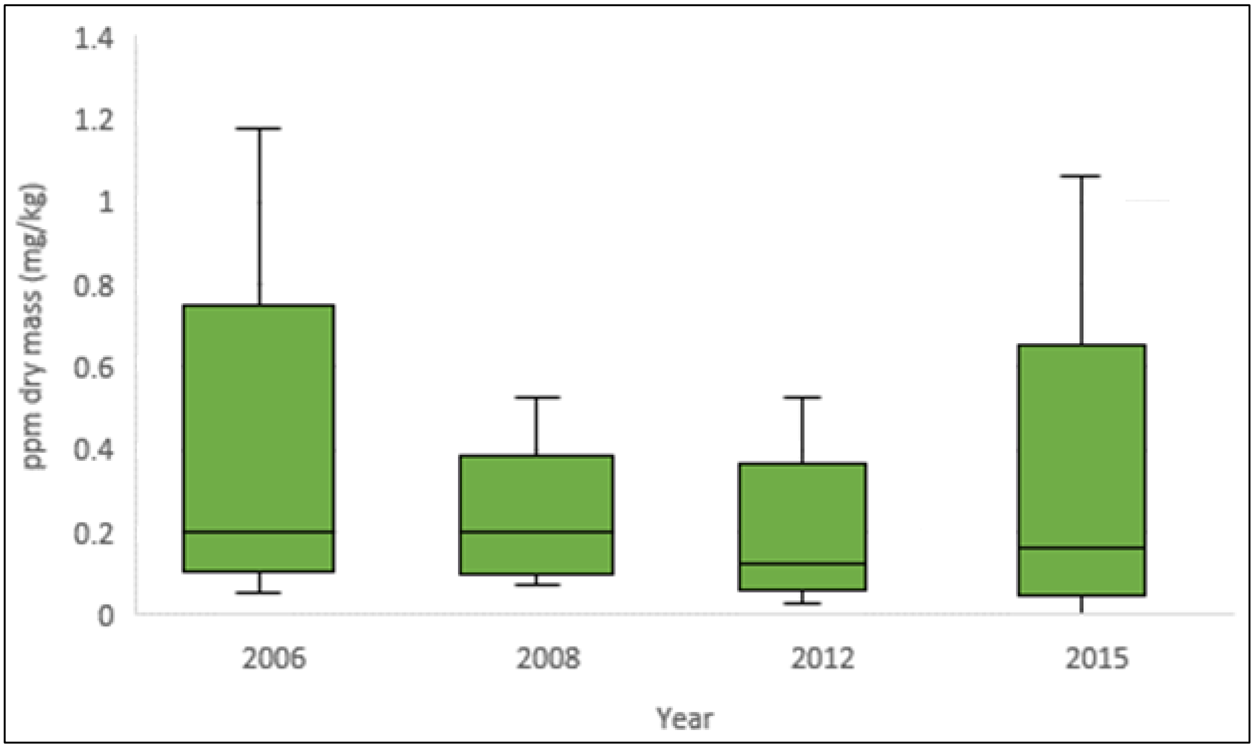


Figure 21: Hydropsychidae accumulation of cadmium from different sampling years in the Rouge River (top error bar: max, top of box: quartile 3, line in box: median, bottom of box: quartile 1, lower error bar: minimum).

Appendix 1: Hydropsychidae Collection Dates and Locations

The ID# labels each unique specimen. See Figure 5 for field identification (FieldID) and River Branch (M, U, Mn, or L).

ID #	Field ID	Branch	Stream Name	Latitude	Longitude	Collection Date	Dry Mass (g)
1	John2	M	Johnson Creek	42.39424	-83.5344	4/9/2006	0.0111
2	John2	M	Johnson Creek	42.39424	-83.5344	4/9/2006	0.0036
3	MR-1	M	Middle Rouge	42.42487	-83.4771	4/29/2006	0.0029
4	MR-1	M	Middle Rouge	42.42487	-83.4771	4/30/2006	0.0029
5	Up1	U	Upper Rouge	42.47526	-83.3857	4/29/2006	0.007
6	Up1	U	Upper Rouge	42.47526	-83.3857	4/29/2006	0.0018
7	Min3	U	Minnow Pond	42.50057	-83.3713	3/18/2006	0.0025
8	Min3	U	Minnow Pond	42.50057	-83.3713	3/18/2006	0.002
9	Main1	MN	Main Rouge	42.60991	-83.1798	5/2/2006	0.0071
10	Main1	MN	Main Rouge	42.60991	-83.1798	5/2/2006	0.0029
11	John6	M	Johnson Creek	42.42546	-83.4814	4/29/2006	0.005
13	See1	U	Seeley Creek	42.51145	-83.4333	4/29/2006	0.0031
14	See1	U	Seeley Creek	42.51145	-83.4333	4/29/2006	0.008
15	Main6	MN	Main Rouge	42.47886	-83.2845	4/29/2006	0.0088
16	Main6	MN	Main Rouge	42.47886	-83.2845	4/29/2006	0.007
17	Up2	U	Upper Rouge	42.46323	-83.3681	4/21/2006	0.0013
18	Up2	U	Upper Rouge	42.46323	-83.3681	4/21/2006	0.004
19	Main4.5	MN	Main Rouge	42.53718	-83.2286	4/29/2006	0.0085
20	Main4.5	MN	Main Rouge	42.53718	-83.2286	4/29/2006	0.0095
21	John1	M	Johnson Creek	42.3897	-83.5819	4/29/2006	0.0063
22	John1	M	Johnson Creek	42.3897	-83.5819	4/29/2006	0.0059
23	Fowl1	L	Fowler Creek	42.30423	-83.6052	4/29/2006	0.0066
24	Fowl1	L	Fowler Creek	42.30423	-83.6052	4/29/2006	0.0027
25	John3	M	Johnson Creek	42.40844	-83.5169	4/29/2006	0.0037
26	John3	M	Johnson Creek	42.40844	-83.5169	4/29/2006	0.005

ID #	Field ID	Branch	Stream Name	Latitude	Longitude	Collection Date	Dry Mass (g)
27	John5	M	Johnson Creek	42.4224	-83.4929	4/29/2006	0.008
28	John5	M	Johnson Creek	42.4224	-83.4929	4/29/2006	0.0101
29	Main4	MN	Main Rouge	42.54242	-83.2248	4/29/2006	0.0106
30	Main4	MN	Main Rouge	42.54242	-83.2248	4/29/2006	0.0088
31	Fowl2	L	Fowler Creek	42.28226	-83.5052	4/29/2006	0.0083
32	Fowl2	L	Fowler Creek	42.28226	-83.5052	4/29/2006	0.006
33	Main5	MN	Main Rouge	42.52219	-83.2469	4/29/2006	0.0022
34	Main5	MN	Main Rouge	42.52219	-83.2469	4/29/2006	0.003
35	Fel1	L	Fellows Creek	42.35729	-83.5399	4/28/2006	0.0021
36	Fel1	L	Fellows Creek	42.35729	-83.5399	4/28/2006	0.0059
37	Main1	MN	Main Rouge	42.60991	-83.1798	4/26/2008	0.0014
38	Main1	MN	Main Rouge	42.60991	-83.1798	4/26/2008	0.0024
39	Main3	MN	Main Rouge	42.54891	-83.2177	4/26/2008	0.0037
40	Main3	MN	Main Rouge	42.54891	-83.2177	4/26/2008	0.0037
41	Main6	MN	Main Rouge	42.47886	-83.2845	4/26/2008	0.0049
42	Main6	MN	Main Rouge	42.47886	-83.2845	4/26/2008	0.0019
43	Up1	U	Upper Rouge	42.47526	-83.3857	4/26/2008	0.0014
44	Up1	U	Upper Rouge	42.47526	-83.3857	4/26/2008	0.0017
45	Ing1	M	Ingersoll Creek	42.46293	-83.4455	4/26/2008	0.0116
46	Ing1	M	Ingersoll Creek	42.46293	-83.4455	4/26/2008	0.0083
47	Wall0	M	Walled Lk Drain	42.43673	-83.4737	4/26/2008	0.0035
48	Wall0	M	Walled Lk Drain	42.43673	-83.4737	4/26/2008	0.0068
49	Wall4	M	Walled Lk Drain	42.43354	-83.4806	4/26/2008	0.0046
50	Wall4	M	Walled Lk Drain	42.43354	-83.4806	4/26/2008	0.0038
51	Wall1	M	Walled Lk Drain	42.44957	-83.4653	4/26/2008	0.0019
52	Wall1	M	Walled Lk Drain	42.44957	-83.4653	4/26/2008	0.0028
53	Up2	U	Upper Rouge	42.46323	-83.3681	4/26/2008	0.0017
54	Up2	U	Upper Rouge	42.46323	-83.3681	4/26/2008	0.0023
55	John3	M	Johnson Creek	42.40844	-83.5169	4/26/2008	0.0038
56	John3	M	Johnson Creek	42.40844	-83.5169	4/26/2008	0.0077
57	Peb2	MN	Pebble Creek	42.51521	-83.344	4/26/2008	0.0033
58	Peb2	MN	Pebble Creek	42.51521	-83.344	4/26/2008	0.0041
59	Peb1	MN	Pebble Creek	42.50133	-83.3291	4/26/2008	0.0031
60	Peb1	MN	Pebble Creek	42.50133	-83.3291	4/26/2008	0.0027

ID #	Field ID	Branch	Stream Name	Latitude	Longitude	Collection Date	Dry Mass (g)
61	Nott	MN	Nottingham Creek	42.51149	-83.2646	4/26/2008	0.0028
62	Nott	MN	Nottingham Creek	42.51149	-83.2646	4/26/2008	0.004
63	John6	M	Johnson Creek	42.42546	-83.4814	4/26/2008	0.0033
64	John6	M	Johnson Creek	42.42546	-83.4814	4/26/2008	0.008
65	Ton1	M	Tonquish Creek	42.36701	-83.499	4/13/2008	0.0041
66	Ton1	M	Tonquish Creek	42.36701	-83.499	4/13/2008	0.0052
67	Fowl2	L	Fowler Creek	42.28226	-83.5052	4/26/2008	0.0053
68	Fowl2	L	Fowler Creek	42.28226	-83.5052	4/26/2008	0.0048
69	John7	M	Johnson Creek	42.39946	-83.5269	4/26/2008	0.0081
70	John7	M	Johnson Creek	42.39946	-83.5269	4/26/2008	0.0095
71	Mid1	M	Middle Rouge	42.42177	-83.4755	4/26/2008	0.0062
72	Mid1	M	Middle Rouge	42.42177	-83.4755	4/26/2008	0.0042
73	Mur2	MN	Murphy Creek	42.59375	-83.2517	4/26/2008	0.0068
74	Mur2	MN	Murphy Creek	42.59375	-83.2517	4/26/2008	0.0043
75	Bell3	U	Bell Branch	42.41015	-83.3929	4/26/2008	0.0098
76	Bell3	U	Bell Branch	42.41015	-83.3929	4/26/2008	0.0052
77	Wall2	M	Walled Lk Drain	42.46732	-83.4662	4/26/2008	0.0095
78	Wall2	M	Walled Lk Drain	42.46732	-83.4662	4/26/2008	0.0051
79	Ton1/2	M	Tonquish Creek	42.36437	-83.4894	4/12/2012	0.0049
80	Ton1/2	M	Tonquish Creek	42.36437	-83.4894	4/12/2012	0.0073
81	Frank2	MN	Franklin Creek	42.52933	-83.3254	4/21/2012	0.0046
82	Frank2	MN	Franklin Creek	42.52933	-83.3254	4/21/2012	0.0025
83	John5	M	Johnson Creek	42.4224	-83.4929	4/21/2012	0.0048
84	John5	M	Johnson Creek	42.4224	-83.4929	4/21/2012	0.0039
85	Fel1	L	Fellows Creek	42.35729	-83.5399	4/21/2012	0.0037
86	Fel1	L	Fellows Creek	42.35729	-83.5399	4/21/2012	0.0082
87	Up1	U	Upper Rouge	42.47526	-83.3857	4/21/2012	0.0052
88	Up1	U	Upper Rouge	42.47526	-83.3857	4/21/2012	0.0082
89	Mid1	M	Middle Rouge	42.42177	-83.4755	4/21/2012	0.0028
90	Mid1	M	Middle Rouge	42.42177	-83.4755	4/21/2012	0.004
91	Main6	MN	Main Rouge	42.47886	-83.2845	4/21/2012	0.003
92	Main6	MN	Main Rouge	42.47886	-83.2845	4/21/2012	0.0088
93	Bish2	M	Bishop Creek	42.47131	-83.4515	4/21/2012	0.0103
94	Bish2	M	Bishop Creek	42.47131	-83.4515	4/21/2012	0.0112

ID #	Field ID	Branch	Stream Name	Latitude	Longitude	Collection Date	Dry Mass (g)
95	Up2	U	Upper Rouge	42.46323	-83.3681	4/21/2012	0.0068
96	Up2	U	Upper Rouge	42.46323	-83.3681	4/21/2012	0.0047
97	John6	M	Johnson Creek	42.42546	-83.4814	4/21/2012	0.0078
98	John6	M	Johnson Creek	42.42546	-83.4814	4/21/2012	0.0062
99	Peb2	MN	Pebble Creek	42.51521	-83.344	4/21/2012	0.0031
100	Peb2	MN	Pebble Creek	42.51521	-83.344	4/21/2012	0.0029
101	Ton1	M	Tonquish Creek	42.36701	-83.499	5/24/2012	0.0082
102	Ton1	M	Tonquish Creek	42.36701	-83.499	5/24/2012	0.0167
103	Wall0	M	Walled Lk Drain	42.43673	-83.4737	4/21/2012	0.0015
104	Wall0	M	Walled Lk Drain	42.43673	-83.4737	4/21/2012	0.002
105	Peb1	MN	Pebble Creek	42.50133	-83.3291	4/21/2012	0.0065
106	Peb1	MN	Pebble Creek	42.50133	-83.3291	4/21/2012	0.0067
107	Min2	U	Minnow Pond	42.49307	-83.3773	4/21/2012	0.0054
108	Min2	U	Minnow Pond	42.49307	-83.3773	4/21/2012	0.0024
109	Frank1	MN	Franklin Creek	42.53024	-83.3059	4/21/2012	0.0068
110	Frank1	MN	Franklin Creek	42.53024	-83.3059	4/21/2012	0.002
111	Main7	MN	Main Rouge	42.47285	-83.2891	4/21/2012	0.0097
112	Main7	MN	Main Rouge	42.47285	-83.2891	4/21/2012	0.0046
113	Main4	MN	Main Rouge	42.54242	-83.2248	4/21/2012	0.0065
114	Main4	MN	Main Rouge	42.54242	-83.2248	4/21/2012	0.0037
115	Wall1	M	Walled Lk Drain	42.44957	-83.4653	4/21/2012	0.0012
116	Wall1	M	Walled Lk Drain	42.44957	-83.4653	4/21/2012	0.0038
117	Peb3	MN	Pebble Creek	42.50085	-83.3245	5/2/2012	0.0078
118	Peb3	MN	Pebble Creek	42.50085	-83.3245	5/2/2012	0.003
119	Main1	MN	Main Rouge	42.60991	-83.1798	4/21/2012	0.0077
120	Main1	MN	Main Rouge	42.60991	-83.1798	4/21/2012	0.0044
121	Nott	MN	Nottingham Creek	42.51149	-83.2646	3/24/2012	0.002
122	Nott	MN	Nottingham Creek	42.51149	-83.2646	3/24/2012	0.0031
123	Bell2	U	Bell Branch	42.41512	-83.429	4/18/2015	0.0089
124	Bell2	U	Bell Branch	42.41512	-83.429	4/18/2015	0.0111
125	Ton1/2	M	Tonquish Creek	42.36437	-83.4894	4/18/2015	0.0016
126	Ton1/2	M	Tonquish Creek	42.36437	-83.4894	4/18/2015	0.0026
127	Min3	U	Minnow Pond	42.50057	-83.3713	4/18/2015	0.0053
128	Min3	U	Minnow Pond	42.50057	-83.3713	4/18/2015	0.0079

ID #	Field ID	Branch	Stream Name	Latitude	Longitude	Collection Date	Dry Mass (g)
129	John6	M	Johnson Creek	42.42546	-83.4814	4/18/2015	0.006
130	John6	M	Johnson Creek	42.42546	-83.4814	4/18/2015	0.0082
131	John1	M	Johnson Creek	42.3897	-83.5819	4/27/2015	0.0104
132	John1	M	Johnson Creek	42.3897	-83.5819	4/27/2015	0.0075
133	Nott	MN	Nottingham Creek	42.51149	-83.2646	4/13/2015	0.01
134	Nott	MN	Nottingham Creek	42.51149	-83.2646	4/13/2015	0.0121
135	Fel4	L	Fellows Creek	42.31346	-83.4647	4/18/2015	0.0072
136	Fel4	L	Fellows Creek	42.31346	-83.4647	4/18/2015	0.0088
137	Peb3	MN	Pebble Creek	42.50085	-83.3245	4/18/2015	0.0045
138	Peb3	MN	Pebble Creek	42.50085	-83.3245	4/18/2015	0.0049
139	John5	M	Johnson Creek	42.4224	-83.4929	4/18/2015	0.0098
140	John5	M	Johnson Creek	42.4224	-83.4929	4/18/2015	0.0081
141	John2	M	Johnson Creek	42.39424	-83.5344	4/18/2015	0.0058
142	John2	M	Johnson Creek	42.39424	-83.5344	4/18/2015	0.0033
143	Bell1	U	Bell Branch	42.42924	-83.3967	10/1/2015	0.0031
144	Bell1	U	Bell Branch	42.42924	-83.3967	10/1/2015	0.0013
145	Mur2	MN	Murphy Creek	42.59375	-83.2517	4/18/2015	0.0118
146	Mur2	MN	Murphy Creek	42.59375	-83.2517	4/18/2015	0.0126
147	Fel5	L	Fellows Creek	42.33526	-83.5398	4/18/2015	0.0009
148	Fel5	L	Fellows Creek	42.33526	-83.5398	4/18/2015	0.0023
149	Fowl1	L	Fowler Creek	42.30423	-83.6052	4/18/2015	0.0068
150	Fowl1	L	Fowler Creek	42.30423	-83.6052	4/18/2015	0.0095
151	Wall3	M	Walled Lk Drain	42.49486	-83.4958	4/18/2015	0.0066
152	Wall3	M	Walled Lk Drain	42.49486	-83.4958	4/18/2015	0.0092
153	Up1	U	Upper Rouge	42.47526	-83.3857	4/18/2015	0.0036
154	Up1	U	Upper Rouge	42.47526	-83.3857	4/18/2015	0.0027
155	John2	M	Johnson Creek	42.39424	-83.5344	4/18/2015	0.003
156	John2	M	Johnson Creek	42.39424	-83.5344	4/18/2015	0.0014
157	Peb1	MN	Pebble Creek	42.50133	-83.3291	4/18/2015	0.0011
158	Peb1	MN	Pebble Creek	42.50133	-83.3291	4/18/2015	0.0029
159	Main4	MN	Main Rouge	42.54242	-83.2248	4/18/2015	0.0111
160	Main4	MN	Main Rouge	42.54242	-83.2248	4/18/2015	0.0081
161	Min2	U	Minnow Pond	42.49307	-83.3773	4/18/2015	0.0042
162	Min2	U	Minnow Pond	42.49307	-83.3773	4/18/2015	0.003

ID #	Field ID	Branch	Stream Name	Latitude	Longitude	Collection Date	Dry Mass (g)
163	Wall0	M	Walled Lk Drainage	42.43673	-83.4737	4/18/2015	0.01
164	Wall0	M	Walled Lk Drainage	42.43673	-83.4737	4/18/2015	0.0037
165	Low3	L	Lower Rouge	42.32365	-83.566	4/18/2015	0.0028
166	Low3	L	Lower Rouge	42.32365	-83.566	4/18/2015	0.0079
169	Main11	MN	Quarton Branch	42.55445	-83.2263	4/18/2015	0.0107
170	Main11	MN	Quarton Branch	42.55445	-83.2263	4/18/2015	0.0073
171	John8	M	Johnson Creek	42.42148	-83.544	4/18/2015	0.0024
172	John8	M	Johnson Creek	42.42148	-83.544	4/18/2015	0.0091
173	LR-5	L	Fellows Creek	42.29451	-83.4358	4/18/2015	0.0035
175	Fel6	L	Fellows Creek	42.32819	-83.5322	4/18/2015	0.0027
176	Fel6	L	Fellows Creek	42.32819	-83.5322	4/18/2015	0.0025
177	MR-23	M	Johnson Creek	42.43248	-83.52	4/16/2015	0.0067
178	MR-23	M	Johnson Creek	42.43248	-83.52	4/16/2015	0.0037
179	Low5	L	Lower Rouge	42.28239	-83.4371	4/18/2015	0.002
180	Low5	L	Lower Rouge	42.28239	-83.4371	4/18/2015	0.0032
181	Mid1	M	Middle Rouge	42.42177	-83.4755	4/18/2015	0.0037
182	Mid1	M	Middle Rouge	42.42177	-83.4755	4/18/2015	0.0003
183	MR-4	M	Middle Rouge	42.36445	-83.4041	4/18/2105	0.004
184	MR-4	M	Middle Rouge	42.36445	-83.4041	4/18/2015	0.0046
185	Ton1	M	Tonquish Creek	42.36701	-83.499	4/18/2015	0.005
186	Ton1	M	Tonquish Creek	42.36701	-83.499	4/18/2015	0.0054

Appendix 2: Hydropsychidae Metal Concentrations (mg/kg dry weight)

ID #	Al27 (ppm)	Cr52 (ppm)	Cr53 (ppm)	Mn55 (ppm)	Co59 (ppm)	Ni60 (ppm)	Cu63 (ppm)	Cu65 (ppm)	Zn66 (ppm)	Zn67 (ppm)
1	167	3.96	3.10	1040	1.16	2.20	24.0	24.0	137.1	133.5
2	351	4.03	2.29	1730	1.10	2.81	29.2	29.1	145.0	145.9
3	1000	5.04	4.13	425	1.21	3.79	37.1	37.7	147.4	146.6
4	457	3.34	2.31	302	0.95	2.16	46.9	47.5	187.9	184.2
5	175	2.36	0.91	1167	0.42	2.26	18.9	19.2	98.6	95.7
6	468	3.85	2.81	2165	0.61	3.48	20.0	20.0	164.8	163.5
7	473	4.62	2.46	459	1.01	2.46	24.3	24.6	220.4	215.8
8	797	5.89	3.08	1526	1.71	3.52	77.3	75.8	305.0	303.2
9	213	2.76	1.32	1018	0.85	3.58	28.4	28.3	144.3	144.2
10	608	3.30	2.35	81	1.02	4.13	32.2	31.8	143.6	145.4
11	405	9.22	7.81	441	0.92	5.10	38.0	37.9	171.9	171.1
13	462	4.54	3.37	443	0.75	5.50	105.4	102.2	246.2	239.9
14	112	4.07	3.37	133	1.46	3.14	32.3	31.8	107.1	104.9
15	252	2.59	1.40	139	0.45	2.20	29.2	29.2	90.8	90.0
16	1126	4.01	3.24	231	1.23	3.90	28.4	28.4	83.0	84.2
17	290	7.28	4.91	1169	1.27	4.15	31.3	31.6	158.3	156.7
18	349	5.72	4.54	1750	1.16	4.02	30.1	30.2	161.6	161.6
19	280	2.42	1.58	456	0.85	2.98	32.7	32.9	155.1	149.7
20	288	2.59	1.93	502	0.85	3.74	29.4	29.1	114.0	111.7
21	96	1.99	0.87	951	2.30	2.74	20.3	20.5	94.0	91.1
22	68	2.41	1.17	772	0.60	2.52	21.4	21.5	87.6	85.8
23	267	2.62	1.43	641	0.65	2.70	22.1	21.7	141.3	139.6
24	680	4.77	2.61	599	1.10	3.99	21.1	21.6	121.0	122.3
25	287	3.86	2.38	2110	2.50	5.14	26.7	27.1	141.2	138.9
26	394	4.33	2.93	2205	2.05	5.85	29.0	30.0	132.0	131.6
27	325	2.60	1.42	433	0.62	2.31	20.6	20.5	94.7	96.4
28	266	0.20	1.09	567	0.63	1.83	21.3	21.7	93.1	91.8

ID #	Al27 (ppm)	Cr52 (ppm)	Cr53 (ppm)	Mn55 (ppm)	Co59 (ppm)	Ni60 (ppm)	Cu63 (ppm)	Cu65 (ppm)	Zn66 (ppm)	Zn67 (ppm)
29	262	0.24	1.50	628	1.23	2.82	37.5	37.9	211.1	209.4
30	169	2.51	1.30	227	0.85	2.94	33.5	33.5	162.1	159.1
31	549	2.90	1.82	928	1.31	3.46	24.0	24.2	99.1	98.1
32	540	2.99	1.91	766	1.30	3.34	23.4	23.5	253.6	250.7
33	501	5.65	4.70	293	1.10	5.90	49.3	48.9	258.8	254.1
34	1394	6.27	5.39	605	1.72	5.83	49.2	49.4	233.3	237.5
35	1693	6.81	5.40	2708	2.36	5.34	32.5	33.1	131.4	129.8
36	733	3.37	2.35	1961	1.36	2.95	17.7	17.5	122.2	124.1
37	368	7.46	4.40	800	1.34	5.42	71.0	71.8	191.3	186.3
38	443	5.50	4.63	612	1.05	4.77	48.4	48.4	150.9	147.4
39	621	4.55	3.89	867	1.66	5.92	33.4	33.4	221.1	220.0
40	509	6.09	4.58	756	2.68	5.71	36.3	37.3	193.7	188.5
41	302	2.96	1.48	302	0.70	2.69	34.6	37.0	127.4	126.7
42	695	6.31	5.27	469	1.33	5.15	53.6	53.4	165.3	166.6
43	1112	6.13	5.58	1699	2.12	5.50	25.6	26.1	134.0	131.8
44	1151	8.09	6.79	2137	2.39	6.21	28.2	29.2	165.8	162.1
45	200	2.51	1.57	1103	1.34	2.55	30.7	31.0	107.7	106.4
46	693	2.94	1.66	1129	1.62	1.99	27.7	28.0	125.7	125.4
47	689	15.40	3.43	949	1.35	3.43	28.1	28.0	198.9	199.9
48	451	3.20	2.26	557	0.95	2.30	20.1	20.3	153.1	153.9
49	302	3.32	2.32	442	0.98	3.49	36.7	36.9	152.9	157.7
50	231	3.44	1.94	466	0.64	2.17	36.2	36.4	160.3	164.8
51	1766	7.82	7.01	872	2.14	5.85	26.9	27.2	130.4	132.9
52	568	4.60	3.77	1441	1.41	3.38	23.7	23.9	135.3	131.6
53	1029	6.86	6.15	1249	2.20	7.12	36.7	37.2	197.7	193.5
54	953	11.96	11.24	1629	2.39	7.99	45.2	45.3	203.0	202.9
55	313	3.56	2.78	1521	1.79	4.83	29.7	29.8	155.7	158.1
56	294	3.00	2.11	1553	1.44	3.20	18.5	18.9	133.5	130.8
57	275	4.23	3.00	2547	1.60	3.37	28.1	28.2	142.2	143.0
58	341	3.57	2.60	1971	1.48	2.98	29.4	30.3	102.1	102.4
59	358	6.03	5.04	1605	1.77	3.87	36.3	36.5	149.5	145.8
60	339	4.24	3.18	2602	1.67	3.83	32.6	32.4	179.1	181.0
61	1480	7.43	6.17	2135	3.38	7.94	28.5	28.7	136.6	137.1
62	882	8.69	7.87	1378	2.45	4.79	26.7	26.3	273.8	271.3

ID #	Al27 (ppm)	Cr52 (ppm)	Cr53 (ppm)	Mn55 (ppm)	Co59 (ppm)	Ni60 (ppm)	Cu63 (ppm)	Cu65 (ppm)	Zn66 (ppm)	Zn67 (ppm)
63	1049	5.37	4.23	922	1.73	5.30	26.4	25.8	119.7	120.0
64	467	3.05	2.37	1304	1.79	3.80	29.3	29.0	118.9	120.5
65	452	3.35	2.20	1139	1.85	3.17	23.8	24.2	136.3	132.9
66	332	2.92	2.20	1344	2.09	3.00	21.3	21.7	135.7	136.0
67	478	2.82	1.97	1387	3.53	3.49	27.4	27.9	131.5	150.8
68	729	3.12	2.52	875	2.86	6.12	83.3	83.5	326.8	340.7
69	219	4.66	3.87	1565	1.52	2.51	23.3	23.8	117.2	123.9
70	232	2.66	1.32	1426	1.22	2.61	22.3	22.4	110.0	113.2
71	330	3.46	2.31	274	0.57	2.77	23.9	24.2	125.8	124.7
72	149	3.67	1.70	149	0.45	2.65	40.1	40.0	119.5	117.0
73	118	2.77	1.26	797	1.39	1.84	33.9	34.5	362.1	354.6
74	187	3.50	1.59	402	0.95	2.20	34.3	33.6	329.2	337.1
75	138	2.42	0.82	262	0.46	1.76	21.1	21.3	94.8	91.6
76	403	3.32	1.44	344	0.74	2.43	19.7	19.7	138.9	138.1
77	248	2.88	1.47	1394	1.07	2.27	26.0	26.4	110.6	110.2
78	323	4.27	2.31	835	0.97	3.54	37.9	38.1	180.8	179.3
79	445	4.02	1.95	1584	1.66	3.43	24.2	24.7	107.8	107.4
80	399	3.00	1.34	1100	1.13	3.19	17.6	17.4	100.5	100.3
81	260	3.66	1.65	1114	1.58	3.06	40.2	39.9	123.9	27.6
82	1053	7.44	4.40	1348	2.24	5.41	42.3	43.2	130.0	129.4
83	174	3.19	1.12	1008	0.99	2.41	22.0	21.6	134.6	130.4
84	187	3.75	1.30	1133	0.65	1.58	20.4	20.8	106.6	106.9
85	420	3.89	1.66	1711	1.31	3.63	42.5	42.4	97.2	95.3
86	343	3.11	1.37	1425	1.13	2.78	33.8	33.9	99.8	113.6
87	235	3.49	1.38	779	0.59	2.09	19.4	19.9	133.6	131.2
88	154	2.79	1.01	600	0.48	1.69	16.8	17.2	83.1	82.5
89	343	4.99	2.16	507	1.22	3.10	34.5	34.1	94.2	99.2
90	627	4.79	2.59	572	1.40	3.49	29.2	29.6	102.1	104.2
91	1058	6.67	3.70	799	1.72	4.91	37.1	37.1	121.6	121.8
92	147	2.56	0.98	277	3.99	2.08	22.6	22.5	76.2	75.2
93	367	2.88	1.41	1192	1.33	2.43	19.6	19.5	112.5	112.6
94	391	3.40	1.89	1199	1.35	2.30	18.5	18.8	125.6	122.5
95	139	3.45	1.33	639	0.47	2.91	26.4	26.5	147.9	148.3
96	350	4.21	1.94	988	1.05	5.24	28.7	28.3	131.9	131.6

ID #	Al27 (ppm)	Cr52 (ppm)	Cr53 (ppm)	Mn55 (ppm)	Co59 (ppm)	Ni60 (ppm)	Cu63 (ppm)	Cu65 (ppm)	Zn66 (ppm)	Zn67 (ppm)
97	409	2.86	1.48	752	0.92	2.24	16.1	16.0	106	104
98	214	3.00	1.03	880	0.87	2.68	20.2	20.8	140	135
99	399	5.54	2.84	1942	1.42	2.66	40.4	40.2	148	156
100	235	4.89	1.90	1337	0.76	2.09	39.9	39.2	103	104
101	351	3.13	1.42	1277	1.70	7.95	18.7	25.0	129	132
102	123	1.75	0.59	616	0.84	2.06	9.6	9.4	64	65
103	501	7.04	3.01	844	1.47	6.16	44.0	44.8	163	167
104	405	5.67	2.42	866	1.21	7.10	39.8	41.0	155	154
105	140	2.93	1.12	760	0.93	2.86	22.5	22.8	139	141
106	103	3.10	1.12	511	0.64	2.27	18.0	18.4	81	82
107	313	3.56	1.59	715	0.81	3.42	20.4	20.6	145	148
108	751	6.23	3.39	472	1.10	3.44	25.0	25.3	142	143
109	320	3.24	1.49	349	1.18	2.52	26.6	26.2	1534	1571
110	364	5.23	2.37	457	1.54	3.14	30.5	30.5	122	126
111	498	3.22	1.77	1028	1.24	2.47	23.4	23.6	110	113
112	451	4.50	2.08	860	1.17	2.87	28.0	27.9	144	144
113	251	3.23	1.35	348	0.54	2.84	22.8	22.6	160	160
114	586	4.88	2.38	539	0.83	4.07	30.5	30.8	220	224
115	1030	8.53	4.40	711	1.10	5.87	35.1	35.0	137	142
116	432	4.60	1.82	502	0.43	2.08	33.2	33.2	146	143
117	330	3.41	1.76	813	1.02	2.81	35.5	36.1	152	161
118	148	4.29	1.21	345	0.37	2.09	31.0	30.9	131	131
119	260	2.89	1.21	941	1.40	2.10	27.1	26.9	111	118
120	114	3.25	1.08	294	0.68	1.90	26.1	25.8	109	112
121	503	6.38	2.26	2836	1.21	3.14	40.6	41.2	131	135
122	508	5.78	2.63	1781	1.17	3.19	45.8	46.0	130	130
123	1152	4.97	3.46	2752	3.23	4.97	27.8	27.6	177	186
124	1007	4.48	3.02	1951	2.43	3.92	28.4	28.8	168	166
125	579	7.49	3.23	936	3.92	3.99	36.0	36.0	179	182
126	802	6.01	2.96	1122	1.27	3.51	37.1	36.8	145	149
127	749	4.75	2.80	8261	4.38	3.57	25.1	25.2	158	169
128	507	4.05	2.27	2562	1.85	3.20	31.6	31.9	160	163
129	250	3.04	1.12	1526	0.97	2.13	17.5	17.9	146	149
130	247	2.40	0.91	1493	0.71	1.97	21.3	21.4	114	117

ID #	Al27 (ppm)	Cr52 (ppm)	Cr53 (ppm)	Mn55 (ppm)	Co59 (ppm)	Ni60 (ppm)	Cu63 (ppm)	Cu65 (ppm)	Zn66 (ppm)	Zn67 (ppm)
131	333	2.55	1.31	1124	0.90	1.82	30.6	30.5	97	100
132	645	2.86	1.60	1257	1.35	2.76	27.0	26.9	142	145
133	568	3.12	1.75	1280	1.05	2.60	28.4	28.0	127	129
134	449	2.89	1.70	1294	1.14	2.50	24.0	24.0	138	139
135	1901	5.21	4.19	1367	3.15	7.23	21.2	21.3	190	194
136	657	3.66	2.16	972	1.73	3.80	22.1	22.7	161	163
137	242	3.67	1.34	1014	0.78	2.22	21.0	21.3	125	125
138	653	4.29	2.63	902	1.17	3.50	21.9	21.8	119	122
139	214	2.71	1.07	1349	0.59	2.54	24.4	24.6	95	96
140	165	2.46	0.72	783	0.62	1.34	16.6	16.4	80	81
141	491	3.87	1.59	1744	1.10	4.49	17.0	17.4	101	103
142	709	4.83	2.07	1091	1.00	3.23	25.2	25.1	60	61
143	1008	5.78	3.16	1129	1.35	4.86	33.0	33.2	140	146
144	2029	15.23	10.07	664	3.55	7.95	64.7	65.1	238	237
145	201	2.93	1.58	4909	2.13	2.08	24.9	25.0	180	185
146	126	2.09	0.91	4056	1.73	1.88	21.6	21.9	121	125
147	925	10.39	3.42	1102	1.22	4.52	51.1	51.2	228	232
148	453	4.30	1.53	779	0.81	2.68	30.0	29.6	100	104
149	315	2.90	1.16	1972	1.07	2.73	23.0	23.1	107	111
150	449	2.40	1.24	2675	1.63	2.63	19.9	19.7	103	107
151	291	2.98	1.47	1917	1.15	1.87	22.2	22.3	124	129
152	146	2.36	0.83	1078	0.66	1.11	17.5	17.3	136	138
153	567	4.46	2.17	758	0.86	2.81	47.1	47.1	175	176
154	712	16.38	13.57	1475	1.87	3.46	40.2	40.8	136	139
155	965	4.69	2.31	1824	1.21	4.62	26.4	26.3	152	160
156	1375	7.46	3.14	961	1.18	5.89	42.7	43.8	146	150
157	462	8.10	2.10	1584	1.80	3.60	44.0	45.2	184	184
158	316	4.48	1.63	1558	2.81	2.84	32.2	31.7	128	131
159	261	2.18	0.97	1283	0.93	2.29	24.7	24.9	94	95
160	404	2.78	1.44	1532	1.22	2.46	25.0	24.9	172	175
161	603	4.40	2.33	1731	1.57	3.46	84.3	84.2	161	165
162	649	4.55	2.20	2601	1.76	3.56	31.2	31.6	136	140
163	196	2.22	0.91	902	0.48	2.66	26.1	26.5	103	104
164	750	5.50	3.00	2615	2.20	3.66	42.3	42.6	152	156

ID #	Al27 (ppm)	Cr52 (ppm)	Cr53 (ppm)	Mn55 (ppm)	Co59 (ppm)	Ni60 (ppm)	Cu63 (ppm)	Cu65 (ppm)	Zn66 (ppm)	Zn67 (ppm)
165	1256	6.21	3.18	3274	3.10	5.70	96.5	99.1	241	248
166	593	3.06	1.71	3761	2.12	3.82	26.9	27.1	113	119
169	157	2.34	1.02	813	0.57	2.80	34.9	35.0	134	135
170	260	2.79	1.31	1046	7.84	2.40	37.6	38.4	163	165
171	1839	12.56	9.99	2179	7.79	7.65	46.0	46.5	169	169
172	1148	4.18	3.05	2621	2.65	6.25	27.5	28.0	167	170
173	1215	5.03	3.14	1594	2.55	7.13	46.9	46.9	134	137
175	1429	5.74	3.59	2111	2.12	6.89	36.5	36.5	142	148
176	937	7.08	4.36	1688	2.24	7.83	29.3	29.6	110	114
177	483	2.71	1.28	4257	1.28	2.82	28.0	28.7	100	106
178	904	4.28	2.17	3020	1.37	4.49	26.2	26.3	136	144
179	1230	7.54	4.40	701	11.61	7.70	63.1	62.2	159	161
180	534	4.95	2.37	520	9.56	4.37	34.4	35.5	143	145
181	341	3.39	1.31	1383	1.10	2.71	31.1	31.3	188	187
182	522	4.83	2.34	1013	0.96	3.26	30.2	30.9	138	137
183	645	4.62	2.70	703	1.32	4.04	35.6	35.7	160	160
184	658	4.78	3.28	1071	0.93	4.50	36.8	36.4	179	177
185	478	2.99	1.67	6299	4.11	3.98	30.9	30.4	164	167
186	521	6.64	5.28	8412	6.74	4.44	29.2	29.1	188	196

ID #	Zn 68 (ppm)	As75 (ppm)	Se77 (ppm)	Se82 (ppm)	Sr88 (ppm)	Cd111 (ppm)	Cd114 (ppm)	Ba135 (ppm)
1	134	1.05	1.14	1.35	21	1.18	1.11	20.8
2	147	1.28	1.38	1.56	17.1	0.21	0.21	52.3
3	146	1.71	1.33	1.25	14	0.15	0.11	38.5
4	186	1.40	1.56	1.40	10.7	0.11	0.11	30.4
5	97	1.05	1.08	1.15	20.3	0.08	0.06	15.5
6	162	1.89	0.24	1.41	22.6	0.18	0.18	31.4
7	217	2.55	1.89	2.55	9.11	0.13	0.13	21.4
8	302	6.16	3.03	2.86	14.3	0.06	0.11	48.0
9	143	2.15	1.69	1.84	29.4	0.22	0.22	30.0
10	144	2.43	1.56	1.48	24.7	0.15	0.23	30.6
11	171	1.30	1.43	1.54	10.5	0.20	0.20	42.1
13	241	1.60	0.78	1.10	44.4	0.46	0.32	22.6
14	105	0.96	0.54	0.74	5.25	0.11	0.10	6.0
15	89	1.35	1.01	1.00	25.7	0.13	0.09	10.8
16	84	2.14	0.97	0.91	34.6	0.17	0.14	22.3
17	157	1.78	1.69	1.86	13.5	0.42	0.34	38.3
18	163	2.20	1.24	1.32	18.4	0.33	0.33	33.5
19	152	1.29	1.79	1.73	14.2	0.17	0.14	15.1
20	114	1.39	1.59	1.60	6.89	0.17	0.15	14.8
21	93	0.45	1.20	1.15	16.6	0.26	0.24	12.7
22	88	0.56	0.99	1.23	14.7	0.22	0.21	13.3
23	141	1.12	1.10	1.07	6.6	0.18	0.17	20.8
24	120	2.40	1.39	1.30	14.4	0.16	0.20	31.3
25	140	1.66	0.92	1.52	30.3	0.42	0.48	34.3
26	130	1.83	1.47	1.61	29.9	0.51	0.44	56.0
27	96	1.28	1.07	1.11	8.48	0.14	0.11	22.0
28	92	1.14	1.05	1.22	18.8	0.12	0.11	23.3
29	210	1.85	1.74	1.94	10.8	0.26	0.24	26.6
30	158	1.25	1.54	1.90	6.06	0.20	0.18	13.9
31	99	1.29	1.66	1.71	16.9	0.21	0.21	28.5
32	252	0.88	1.63	1.67	13.4	0.18	0.20	26.5
33	257	3.95	2.05	2.75	38.3	0.95	1.05	15.7
34	235	4.33	1.43	1.54	64.4	0.92	0.95	120.0
35	129	3.40	1.31	1.78	29.4	0.31	0.42	45.8

ID #	Zn 68 (ppm)	As75 (ppm)	Se77 (ppm)	Se82 (ppm)	Sr88 (ppm)	Cd111 (ppm)	Cd114 (ppm)	Ba135 (ppm)
36	123	1.79	1.14	1.51	21.9	0.30	0.28	36.0
37	190	5.26	1.73	2.59	72.9	0.24	0.31	38.0
38	148	4.54	1.93	1.56	57.2	0.18	0.18	33.5
39	221	4.61	1.96	2.11	28.2	0.21	0.24	50.1
40	189	4.58	1.84	2.38	27.5	0.18	0.00	43.7
41	128	1.84	0.85	0.97	18.4	0.18	0.18	25.0
42	167	3.18	0.81	1.10	16.6	0.12	0.12	37.3
43	133	2.83	0.94	1.73	24.8	0.16	0.24	45.5
44	162	2.72	1.29	1.75	24.6	0.32	0.19	32.6
45	108	2.01	1.41	1.65	18.3	0.21	0.13	27.9
46	125	2.49	1.83	1.84	16.5	0.20	0.20	49.8
47	199	2.61	1.98	2.04	17.8	0.22	0.25	36.5
48	157	1.81	1.93	1.96	28.7	0.16	0.15	25.6
49	84	1.12	1.32	1.46	11.3	0.22	0.14	96.3
50	162	0.75	1.27	1.22	10.2	0.12	0.14	68.6
51	132	3.24	0.75	1.39	31.3	0.17	0.23	45.2
52	135	1.69	1.10	1.02	21.6	0.12	0.12	24.4
53	193	2.33	0.32	0.91	35.5	0.13	0.19	34.0
54	200	2.77	0.77	1.34	38.3	0.24	0.24	56.2
55	156	1.88	1.24	1.77	36.5	0.43	1.16	50.6
56	133	3.54	1.17	1.51	32.6	0.20	0.21	46.3
57	143	2.53	2.00	2.30	24.5	0.17	0.13	37.8
58	100	2.28	1.85	2.12	26.4	0.08	0.11	38.6
59	147	2.06	1.45	1.92	24.7	0.07	0.11	26.9
60	179	1.83	1.39	1.79	26.4	0.12	0.16	37.0
61	138	5.62	2.32	2.67	30.4	0.24	0.31	62.5
62	278	4.10	2.09	3.11	26.1	0.22	0.19	40.8
63	121	3.20	1.17	1.53	18.7	0.20	0.17	36.6
64	120	3.20	1.77	1.87	15.7	0.17	0.15	50.5
65	132	2.95	1.99	1.99	14.4	0.46	0.48	30.7
66	138	2.73	1.59	1.73	13	0.53	0.53	40.0
67	147	1.10	1.39	1.27	37.5	0.27	0.27	330.0
68	341	1.12	1.38	1.54	84	0.28	0.28	315.4
69	123	2.61	1.24	1.53	40.9	0.22	0.22	120.5

ID #	Zn 68 (ppm)	As75 (ppm)	Se77 (ppm)	Se82 (ppm)	Sr88 (ppm)	Cd111 (ppm)	Cd114 (ppm)	Ba135 (ppm)
70	111	2.88	1.39	1.46	41.5	0.34	0.32	76.9
71	124	1.58	1.19	1.53	23.1	0.11	0.09	21.8
72	119	1.02	1.28	1.41	9.35	0.08	0.10	8.2
73	358	20.37	2.70	3.30	14.8	0.24	0.24	99.4
74	333	12.92	2.92	3.40	10.6	0.23	0.26	174.4
75	94	1.20	1.63	1.71	20.3	0.08	0.08	10.6
76	139	1.18	1.14	1.27	10.4	0.13	0.13	21.7
77	111	1.45	0.98	0.90	15.2	0.19	0.17	34.2
78	178	0.86	1.08	1.04	20.7	0.28	0.30	22.2
79	107	1.86	1.28	1.64	27.3	0.20	0.20	40.3
80	102	1.15	1.39	1.69	17.1	0.18	0.20	19.5
81	123	1.24	1.10	1.51	10.7	0.33	0.38	20.0
82	131	2.11	0.88	1.36	17.9	0.53	0.62	33.3
83	134	1.26	0.85	11.46	24.5	0.14	0.16	34.2
84	108	1.55	0.79	1.04	28.5	0.06	0.11	38.7
85	97	1.69	0.80	0.98	26.8	0.21	0.27	29.0
86	110	1.42	0.82	0.82	38.1	0.25	0.27	214.7
87	134	0.74	0.61	0.97	27.1	0.08	0.13	31.2
88	83	0.85	0.75	0.79	10.7	0.05	0.07	16.1
89	97	2.36	1.93	2.32	18.7	0.20	0.24	115.3
90	106	2.75	1.73	2.01	19.8	0.22	0.28	53.0
91	122	1.83	1.25	1.47	36	0.11	0.15	30.9
92	76	0.51	0.90	1.06	14.2	0.08	0.08	8.6
93	114	1.08	1.92	2.01	52.1	0.16	0.17	24.5
94	124	1.21	1.59	1.69	56.5	0.09	0.11	20.3
95	150	0.61	0.54	0.76	158	0.13	0.15	39.4
96	131	0.98	0.54	0.94	25.9	0.28	0.23	35.0
97	106	0.97	1.00	1.07	12.4	0.06	0.10	24.9
98	137	0.71	0.99	0.92	13	0.18	0.20	22.3
99	154	2.02	1.60	1.56	32.4	0.21	0.28	126.6
100	104	1.29	1.25	1.29	37.4	0.04	0.11	24.8
101	132	2.20	2.39	2.03	13.5	0.23	0.21	30.9
102	64	1.17	1.65	1.64	17.1	0.10	0.09	11.2
103	164	1.03	2.20	0.95	49.1	0.15	0.15	27.4

ID #	Zn 68 (ppm)	As75 (ppm)	Se77 (ppm)	Se82 (ppm)	Sr88 (ppm)	Cd111 (ppm)	Cd114 (ppm)	Ba135 (ppm)
104	156	1.38	1.38	1.32	43.5	0.11	0.17	22.2
105	142	1.29	2.22	1.98	16.9	0.20	0.22	28.4
106	80	1.54	1.77	2.05	25.8	0.11	0.13	11.4
107	148	1.34	1.63	1.59	13.1	0.12	0.12	92.5
108	141	1.74	2.70	1.97	31.6	0.05	0.09	25.3
109	1564	3.11	2.62	2.48	30.6	0.15	0.16	15.7
110	128	2.48	3.08	2.48	10.5	0.17	0.22	37.4
111	111	2.36	2.05	2.31	38.5	0.09	0.10	57.1
112	143	0.98	2.22	1.53	28.6	0.12	0.12	37.7
113	159	1.68	2.25	2.03	53.7	0.15	0.14	22.9
114	224	2.59	2.32	2.20	81.5	0.21	0.21	56.4
115	139	1.28	2.11	1.38	47.9	0.09	0.18	39.9
116	144	0.87	1.59	1.39	38.5	0.03	0.06	20.3
117	158	1.28	2.14	2.64	33.6	0.20	0.23	92.8
118	133	0.73	2.24	1.94	36.3	0.07	0.07	12.7
119	118	3.60	1.81	1.80	11.1	0.09	0.09	93.9
120	108	1.78	2.78	2.00	6.9	0.10	0.10	10.4
121	139	2.70	3.30	2.75	42.2	0.06	0.11	64.9
122	131	1.81	2.70	2.24	42.6	0.11	0.11	39.7
123	186	3.51	3.67	3.76	22.6	0.22	0.16	71.7
124	167	3.05	3.17	3.17	25.5	0.19	0.15	54.4
125	182	1.93	2.54	1.51	33.3	0.07	0.14	39.5
126	149	2.45	2.54	1.90	36.9	0.21	0.17	49.7
127	171	4.28	1.99	2.05	26.8	0.19	0.15	169.0
128	162	2.45	1.91	2.13	19.5	0.18	0.14	62.6
129	146	2.09	1.30	1.14	24.1	0.06	0.09	44.5
130	117	1.68	1.17	1.10	20.3	0.08	0.09	35.3
131	98	0.78	1.07	1.10	11.7	0.10	0.10	29.4
132	147	0.85	1.04	0.98	20.9	0.19	0.19	42.2
133	127	2.08	1.83	1.73	25.9	0.20	0.20	29.4
134	138	1.94	1.82	1.63	27.5	0.17	0.18	33.2
135	194	2.64	2.64	2.61	54.2	0.32	0.28	61.3
136	163	1.46	2.18	2.20	42.9	0.23	0.21	32.1
137	125	1.76	1.39	1.49	19.5	0.12	0.12	43.0

ID #	Zn 68 (ppm)	As75 (ppm)	Se77 (ppm)	Se82 (ppm)	Sr88 (ppm)	Cd111 (ppm)	Cd114 (ppm)	Ba135 (ppm)
138	121	2.38	1.26	1.21	67.8	0.09	0.11	44.0
139	97	2.09	1.56	1.62	21.2	0.10	0.11	36.5
140	81	1.06	0.96	0.99	18.4	0.10	0.10	16.4
141	104	1.82	1.04	1.06	32.1	0.28	0.28	57.3
142	261	1.20	0.30	0.27	26.8	0.10	0.20	49.6
143	143	2.80	1.45	1.31	42	0.14	0.18	59.2
144	243	2.96	2.37	1.44	46.4	0.25	0.34	57.4
145	181	12.33	2.01	2.32	33.2	0.17	0.16	88.4
146	124	6.72	2.04	2.03	22	0.10	0.10	63.1
147	228	1.22	1.10	0.37	19.4	0.37	0.37	34.0
148	101	1.15	1.00	0.10	11.3	0.00	0.10	21.9
149	108	2.09	1.02	0.99	10.9	0.10	0.13	44.0
150	105	4.24	0.98	1.01	12.8	0.19	0.19	66.7
151	127	2.05	1.03	0.83	50.4	0.07	0.08	59.1
152	139	1.00	0.71	0.60	33.4	0.01	0.04	25.0
153	177	1.34	1.50	1.16	42.6	0.06	0.09	29.2
154	137	1.87	1.67	0.94	41.8	0.04	0.12	39.0
155	157	2.16	1.03	0.95	50.9	0.11	0.18	90.2
156	149	1.81	1.18	0.79	39.8	0.08	0.24	81.9
157	185	1.20	2.80	0.90	32.4	0.20	0.40	43.9
158	130	1.33	1.44	1.82	28.3	0.27	0.30	34.7
159	94	1.27	1.37	1.40	8.8	0.06	0.06	29.5
160	171	2.15	1.66	1.43	13.8	0.16	0.12	40.7
161	164	1.91	1.00	0.92	20.5	0.10	0.18	49.0
162	137	2.05	1.39	0.92	14.6	0.11	0.15	60.3
163	103	0.63	1.22	1.19	20.4	0.06	0.07	22.7
164	154	2.05	1.69	1.46	46.8	0.24	0.30	75.2
165	241	6.13	1.81	0.86	29.1	0.12	0.16	101.0
166	117	4.51	1.03	0.97	19.4	0.14	0.13	95.5
169	134	1.06	1.88	1.99	24.8	0.10	0.11	26.3
170	163	1.91	1.64	1.73	21.9	0.27	0.30	39.0
171	172	2.06	1.83	1.38	23.8	0.41	0.46	52.3
172	171	1.63	1.20	1.44	19.5	0.34	0.36	60.1
173	136	1.57	1.48	1.60	37.3	0.25	0.22	39.4

ID #	Zn 68 (ppm)	As75 (ppm)	Se77 (ppm)	Se82 (ppm)	Sr88 (ppm)	Cd111 (ppm)	Cd114 (ppm)	Ba135 (ppm)
175	145	2.85	1.91	1.18	46.8	0.24	0.24	53.3
176	110	2.20	2.07	1.19	43.9	0.35	0.35	33.4
177	105	2.02	1.79	1.64	13.1	0.18	0.18	88.5
178	142	2.71	1.93	1.49	17.1	0.24	0.33	92.5
179	163	1.05	1.93	1.16	19.2	0.17	0.22	19.2
180	143	0.45	1.72	1.00	17.7	0.00	0.07	11.6
181	190	1.75	1.37	1.04	14.7	0.09	0.09	39.2
182	137	1.70	1.95	1.28	17.2	0.11	0.14	28.2
183	158	1.13	1.38	0.91	25.7	0.11	0.14	25.7
184	177	1.32	1.48	1.41	31	0.29	0.19	34.8
185	170	6.12	0.92	1.30	32.6	0.22	0.33	149.5
186	192	6.80	1.81	1.96	36.9	0.37	0.33	180.7

ID #	Ba137 (ppm)	Pb208 (ppm)	Na23 (ppm)	Mg24 (ppm)	K39 (ppm)	Ca44 (ppm)	Fe54 (ppm)
1	20.5	12.40	2910	1380	2361	5196	1282
2	51.7	2.63	3210	1205	2712	5431	1618
3	38.9	5.16	2897	1989	3456	6305	2715
4	30.0	4.17	7796	1357	2987	3742	1523
5	15.5	1.56	1880	915	1961	3592	906.8
6	32.0	4.77	6438	1522	3085	6316	1910
7	21.5	4.58	9514	1204	2545	3096	1648
8	46.9	6.60	4663	1421	3142	3426	2441
9	30.0	2.18	1515	1685	456.4	16042	1298
10	0.8	2.35	3668	1647	503.3	14768	2080
11	42.5	3.98	5451	1085	2564	3213	1500
13	22.7	13.98	1E+05	4764	4616	12494	2322
14	5.9	1.33	3063	664.6	2466	1075	766.4
15	10.6	1.98	2012	1038	931.4	7067	865.8
16	22.2	4.35	1247	2206	1249	11711	3140
17	37.5	4.91	4946	1306	4032	4662	3018
18	34.1	4.07	9750	1541	4945	6958	1401
19	15.1	2.81	6541	1501	3685	4876	1172
20	14.4	2.57	2835	1211	2966	3641	1532
21	12.8	1.45	3244	811.4	1730	4485	479.8
22	13.4	1.45	4802	653.5	1500	3916	1028
23	20.8	2.08	2381	1053	2035	2368	2081
24	30.9	3.54	3528	1671	2819	4728	5368
25	34.2	2.59	3337	1163	1790	7301	2433
26	53.2	2.60	2212	1198	2067	7624	2807
27	21.9	1.86	2107	835.4	2708	2516	1944
28	23.2	1.47	1456	1109	3138	3867	1602
29	26.7	2.87	2503	1316	3706	3122	1557
30	13.8	2.25	3528	804.5	2937	1721	943
31	28.4	2.11	1577	1284	2073	3151	1824
32	26.4	2.27	5633	1240	2011	2911	1757
33	15.7	5.00	2370	1400	446.5	17278	2888
34	121.7	8.62	1801	3204	979.6	25272	5164
35	46.2	5.50	3119	1493	2395	6849	7667

ID #	Ba137 (ppm)	Pb208 (ppm)	Na23 (ppm)	Mg24 (ppm)	K39 (ppm)	Ca44 (ppm)	Fe54 (ppm)
36	35.8	2.80	1810	896.2	1904	4287	3627
37	37.8	4.16	2742	3427	693.2	17292	3631
38	33.6	3.44	4222	2743	685.3	14256	4671
39	49.0	4.13	3974	2571	2835	12354	4216
40	43.5	3.98	3481	2067	2549	11622	4063
41	24.8	3.64	12542	2280	2326	7019	1142
42	37.1	5.21	4369	2736	3088	10189	4785
43	45.6	7.46	4088	2526	3606	9883	4967
44	32.3	7.25	4742	2227	4539	8262	5557
45	27.9	2.50	2991	1077	2657	3029	1754
46	50.0	9.28	3419	1147	2667	2919	1647
47	35.8	4.62	3904	1258	3380	4160	3245
48	25.7	2.99	2496	1048	2701	4775	1941
49	94.3	3.56	5178	1052	3259	2913	1720
50	68.3	3.44	3766	1061	2965	2419	944.4
51	45.7	8.11	4438	2153	3207	9322	6809
52	24.5	3.65	5625	1618	3169	5298	3140
53	34.2	8.02	4503	2844	2290	14534	5385
54	54.9	126.83	5757	2650	3056	14548	6743
55	50.3	3.59	4165	1264	1822	6800	3712
56	46.2	2.40	990.6	1001	141.1	5465	4471
57	47.6	3.73	4838	1109	2408	4845	3053
58	38.5	3.38	2590	1124	1993	5136	2916
59	26.5	3.69	4254	1363	3858	5444	4072
60	36.9	3.75	10650	1824	3741	6897	2390
61	62.3	8.76	3952	2041	3198	12055	6864
62	40.5	8.03	4391	1589	3350	7464	4346
63	36.1	5.30	4230	2448	3984	8927	4547
64	50.9	3.12	3037	1493	3110	4112	2774
65	30.5	2.55	4016	1081	3016	3817	2994
66	40.5	2.24	4618	1038	4176	3356	2770
67	333.8	1.95	1971	1301	1325	9887	1563
68	316.3	14.64	6097	2490	1579	16747	2771
69	120.7	2.06	1065	939.4	694.8	6232	2449

ID #	Ba137 (ppm)	Pb208 (ppm)	Na23 (ppm)	Mg24 (ppm)	K39 (ppm)	Ca44 (ppm)	Fe54 (ppm)
70	77.1	2.99	1552	1078	880.1	6460	2164
71	21.8	2.96	5514	1301	2554	4858	1439
72	8.3	3.12	2576	814.7	2131	2550	1067
73	99.9	5.73	2393	834.9	1816	3642	1980
74	171.0	6.22	2606	865.1	2289	3665	2120
75	10.8	1.78	1904	793.4	1873	3143	613.1
76	21.7	2.45	2891	1209	2496	3459	1195
77	34.7	3.49	1671	969.3	1821	2580	1656
78	22.6	5.03	2454	1321	2393	5391	1603
79	40.3	2.36	2499	1244	2560	11953	1960
80	19.5	1.55	1694	1160	2336	6759	1519
81	20.0	2.56	2636	1031	2761	3420	1787
82	33.3	5.54	2930	2018	3049	8016	4411
83	33.9	1.79	1859	927.5	1147	5606	1343
84	39.0	1.95	1897	864.4	882	5428	1598
85	29.2	2.05	2943	1008	2400	4026	2105
86	214.4	1.44	2398	1003	2676	3527	2001
87	31.6	1.97	2605	1049	1914	4852	1377
88	16.0	1.33	1543	779.5	1515	2692	1289
89	115.3	38.34	4012	1059	3192	4039	1715
90	53.2	42.98	4180	1382	3344	5775	3035
91	30.7	5.72	2865	2684	1745	18422	4164
92	8.6	1.21	1211	839.8	1048	6722	672.1
93	24.4	2.51	2006	1268	2470	3903	1658
94	20.4	2.95	2057	1281	2461	4326	1901
95	39.5	2.02	2729	1263	2809	4846	755
96	34.5	2.86	3977	1623	4304	9276	1759
97	25.0	2.10	1651	1188	2254	4079	1912
98	22.3	1.79	2233	1171	2686	3189	1045
99	124.7	3.65	2600	1619	2409	8559	3370
100	24.8	2.35	2130	1110	2033	6234	2145
101	31.1	1.46	3033	829.9	2690	4023	1848
102	11.2	0.51	1593	744.1	1729	3170	759.7
103	27.4	3.23	7524	1292	1752	10471	1425

ID #	Ba137 (ppm)	Pb208 (ppm)	Na23 (ppm)	Mg24 (ppm)	K39 (ppm)	Ca44 (ppm)	Fe54 (ppm)
104	22.2	2.86	6074	1273	2266	8620	1314
105	28.4	1.12	2673	821.7	2444	3923	929.6
106	11.4	0.95	2502	694.9	1995	3849	929
107	92.5	1.81	2500	1055	1995	3497	1460
108	25.3	3.94	2744	1732	1994	7952	2925
109	15.7	1.76	3971	1489	2108	21719	1841
110	37.2	2.59	5143	1112	2622	3936	2025
111	56.9	2.84	1385	1650	1903	15328	2269
112	37.7	2.68	2775	1417	1817	11379	1666
113	22.5	2.30	1658	1645	775.2	19507	1190
114	56.3	4.64	3211	2019	1100	26108	2532
115	38.9	4.58	6027	1849	2385	11606	3358
116	20.5	2.00	4457	1141	3386	7229	1347
117	91.7	2.40	2916	1978	2504	6918	1710
118	12.9	1.43	2922	1117	1849	5851	785.6
119	94.7	1.16	2343	1387	2993	6405	1971
120	10.3	1.05	2776	608.9	1915	2664	740.2
121	65.8	3.36	2672	1370	1958	13960	2607
122	42.5	2.66	2944	1370	1824	11805	2299
123	72.1	3.87	2704	2082	4172	6135	4311
124	54.9	3.28	2324	1892	3786	6127	3695
125	39.3	3.44	12204	1446	2585	9716	1996
126	49.9	2.79	4657	1539	2821	13661	2922
127	169.1	3.86	3039	1531	3599	4539	4206
128	61.6	3.19	3195	1597	3035	4376	2779
129	44.8	2.05	4125	998.8	1461	4790	1776
130	35.2	1.64	2375	896.7	1391	4205	1487
131	29.2	0.97	2170	1288	1988	3489	1733
132	42.1	2.20	3112	2015	2343	10518	2706
133	28.8	2.44	3781	1433	3184	5761	3175
134	32.9	2.07	3735	1344	3207	5443	2751
135	61.9	3.93	4685	2957	3193	12879	4754
136	32.3	2.04	4560	1731	3059	8688	2092

ID #	Ba137 (ppm)	Pb208 (ppm)	Na23 (ppm)	Mg24 (ppm)	K39 (ppm)	Ca44 (ppm)	Fe54 (ppm)
137	43.5	2.00	5799	927.8	2562	3312	1921
138	43.9	3.14	5685	2486	2697	17978	3679
139	36.2	1.01	4930	881.3	1582	5569	1608
140	16.4	0.83	2178	661.2	824.3	4938	975.7
141	58.8	2.66	4949	1047	2265	6047	2910
142	50.0	3.17	5035	1140	1988	6080	2794
143	59.0	3.02	9869	1796	3297	9893	3426
144	57.3	5.67	10768	2799	2590	12655	5997
145	89.4	3.23	3041	1407	2442	7966	5056
146	63.4	1.75	2450	1059	1775	5139	2556
147	34.5	3.42	23328	1316	2131	4037	2152
148	21.4	1.72	17121	1191	2886	2531	1599
149	44.1	4.69	4029	1213	2730	2740	4662
150	65.9	1.39	2832	1340	2693	2718	10496
151	59.0	2.88	5095	752.7	2590	6058	2406
152	25.2	1.46	4749	539.4	1922	4654	1381
153	29.0	2.93	7786	1774	3213	7225	2675
154	38.5	4.16	7553	1740	2694	8049	3074
155	88.9	3.74	7656	1865	4419	6770	3981
156	83.6	4.87	18112	1783	3634	6803	4328
157	44.4	3.30	11079	1007	1906	7250	1592
158	34.9	2.20	7100	1156	1361	6799	1578
159	29.9	1.74	2628	1086	2977	2707	1362
160	41.1	3.03	3562	1417	3286	5896	2266
161	48.5	4.27	5414	1513	2961	4550	2931
162	60.5	3.19	9950	1434	3445	3302	2552
163	22.7	1.18	3871	917.3	2492	2827	796
164	75.3	3.42	12122	1407	2729	7313	2903
165	102.3	3.97	11914	1927	4180	5347	7834
166	94.9	1.87	3833	1302	3189	2906	5091
169	25.9	2.24	4270	1402	2669	7004	1134
170	39.4	3.78	5501	1191	2099	7544	1754
171	53.4	9.53	11292	1895	2817	6797	5251

ID #	Ba137 (ppm)	Pb208 (ppm)	Na23 (ppm)	Mg24 (ppm)	K39 (ppm)	Ca44 (ppm)	Fe54 (ppm)
172	61.1	3.67	3879	1783	3075	5610	3527
173	39.0	4.21	5144	2357	2722	8732	3451
175	53.7	3.95	8456	2743	2455	11515	4451
176	33.4	2.95	10090	1875	1990	10220	2942
177	90.0	2.04	4542	853.3	2944	6335	2124
178	95.0	3.03	9649	1263	4409	9970	3823
179	19.0	4.57	26437	1564	3107	4453	2786
180	11.8	2.13	6456	834	2191	2042	1031
181	39.5	2.68	7110	845.6	1841	3371	1237
182	29.1	4.47	10144	1122	2031	3975	1785
183	25.5	4.29	8239	2028	2214	7960	2278
184	34.4	5.05	6488	1761	3116	6882	2395
185	147.1	2.29	11870	1078	2495	8615	4237
186	176.4	2.34	9158	1176	2579	9501	4275

Appendix 3: Benthic Invertebrate Biodiversity Scores

The Stream Quality Score (SQI) and number of taxa (orders) are measures of benthic invertebrate stream biodiversity and richness calculated using the following identification and assessment procedures. This data was provided by Friends of the Rouge and can be found following the identification and assessment procedure. See figure 5.

IDENTIFICATION AND ASSESSMENT

Use letter codes [**R** (rare) = 1-10, **C** (common) = 11 or more] to record the approximate numbers of organisms in each taxa found in the stream reach.

*** Do NOT count empty shells, pupae, or terrestrial macroinvertebrates***

Group 1: Sensitive

____ Caddisfly larvae (Trichoptera)
EXCEPT Net-spinning caddis
____ Hellgrammites (Megaloptera)
____ Mayfly nymphs (Ephemeroptera)
____ Gilled (right-handed) snails (Gastropoda)
____ Stonefly nymphs (Plecoptera)
____ Water penny (Coleoptera)
____ Water snipe fly (Diptera)

Group 2: Somewhat-Sensitive

____ Alderfly larvae (Megaloptera)
____ Beetle adults (Coleoptera)
____ Beetle larvae (Coleoptera)
____ Black fly larvae (Diptera)
____ Clams (Pelecypoda)
____ Crane fly larvae (Diptera)
____ Crayfish (Decapoda)
____ Damselfly nymphs (Odonata)
____ Dragonfly nymphs (Odonata)
____ Net-spinning caddisfly larvae (Hydropsychidae; Trichoptera)
____ Scuds (Amphipoda)
____ Sowbugs (Isopoda)

Group 3: Tolerant

____ Aquatic worms (Oligochaeta)
____ Leeches (Hirudinea)
____ Midge larvae (Diptera)
____ Pouch snails (Gastropoda)
____ True bugs (Hemiptera)
____ Other true flies (Diptera)

STREAM QUALITY SCORE

Group 1:
____ # of R's * 5.0 = ____
____ # of C's * 5.3 = ____
Group 1 Total = ______

Group 2:
____ # of R's * 3.0 = ____
____ # of C's * 3.2 = ____
Group 2 Total = ______

Group 3:
____ # of R's * 1.1 = ____
____ # of C's * 1.0 = ____
Group 3 Total = ______

Total Stream Quality Score = _______
(Sum of totals for groups 1-3; round to nearest whole number)

Check one:
____ Excellent (>48)
____ Good (34-48)
____ Fair (19-33)
____ Poor (<19)

FIELDID	SQI	# Taxa	Latitude	Longitude	Collection Date
Bell1	23	9	42.42924	-83.3967	10/17/2015
Bell2	30	13	42.41512	-83.429	4/18/2015
Bell3	14	6	42.41015	-83.3929	4/26/2008
Bish2	21	8	42.47131	-83.4515	4/21/2012
Fel1	36	14	42.35729	-83.5399	4/28/2006
Fel1	26	9	42.35729	-83.5399	4/21/2012
Fel4	33	15	42.31346	-83.4647	4/18/2015
Fel5	41	13	42.33526	-83.5398	4/18/2015
Fel6	25	8	42.32819	-83.5322	4/18/2015
Fowl1	45	18	42.30423	-83.6052	4/29/2006
Fowl1	42	19	42.30423	-83.6052	4/18/2015
Fowl2	28	8	42.28226	-83.5052	4/29/2006
Fowl2	33	12	42.28226	-83.5052	4/26/2008
Frank1	33	14	42.53024	-83.3059	4/21/2012
Frank2	24	10	42.52933	-83.3254	4/21/2012
Ing1	24	12	42.46293	-83.4455	4/26/2008
John1	55	20	42.3897	-83.5819	4/29/2006
John1	52	21	42.3897	-83.5819	4/27/2015
John2	53	20	42.39424	-83.5344	4/9/2006
John2	46	17	42.39424	-83.5344	4/18/2015
John2	46	17	42.39424	-83.5344	4/18/2015
John3	37	12	42.40844	-83.5169	4/29/2006
John3	39	15	42.40844	-83.5169	4/26/2008
John5	21	8	42.4224	-83.4929	4/29/2006
John5	28	10	42.4224	-83.4929	4/21/2012
John5	34	12	42.4224	-83.4929	4/18/2015
John6	20	9	42.42546	-83.4814	4/29/2006
John6	15	6	42.42546	-83.4814	4/26/2008
John6	35	14	42.42546	-83.4814	4/21/2012
John6	26	11	42.42546	-83.4814	4/18/2015
John7	46	19	42.39946	-83.5269	4/26/2008
John8	36	14	42.42148	-83.544	4/18/2015
Low3	33	12	42.32365	-83.566	4/18/2015
Low5	30	11	42.28239	-83.4371	4/18/2015
LR-5	18	9	42.29451	-83.4358	4/18/2015

FIELDID	SQI	# Taxa	Latitude	Longitude	Collection Date
Main1	40	14	42.6099	-83.18	5/2/2006
Main1	19	10	42.6099	-83.18	4/26/2008
Main1	29	13	42.6099	-83.18	4/21/2012
Main11	36	13	42.5545	-83.226	4/18/2015
Main3	30	10	42.5489	-83.218	4/26/2008
Main4	28	9	42.5424	-83.225	4/29/2006
Main4	34	11	42.5424	-83.225	4/21/2012
Main4	23	9	42.5424	-83.225	4/18/2015
Main4.5	32	12	42.5372	-83.229	4/29/2006
Main5	23	12	42.5222	-83.247	4/29/2006
Main6	17	8	42.4789	-83.285	4/29/2006
Main6	18	8	42.4789	-83.285	4/26/2008
Main6	40	14	42.4789	-83.285	4/21/2012
Main7	32	12	42.4729	-83.289	4/21/2012
Mid1	20	8	42.4218	-83.476	4/26/2008
Mid1	41	19	42.4218	-83.476	4/21/2012
Mid1	19	7	42.4218	-83.476	4/18/2015
Min2	36	15	42.4931	-83.377	4/21/2012
Min2	30	12	42.4931	-83.377	4/18/2015
Min3	18	7	42.5006	-83.371	4/18/2015
MR-1	32	12	42.4249	-83.477	4/30/2006
MR-23	42	17	42.4325	-83.52	4/16/2015
MR-4	21	9	42.3645	-83.404	4/18/2015
MR-4	21	9	42.3645	-83.404	4/18/2105
Mur2	23	10	42.5938	-83.252	4/26/2008
Mur2	34	14	42.5938	-83.252	4/18/2015
Nott	29	12	42.5115	-83.265	4/26/2008
Nott	22	9	42.5115	-83.265	4/13/2015
Peb1	22	9	42.5013	-83.329	4/26/2008
Peb1	24	9	42.5013	-83.329	4/21/2012
Peb1	27	10	42.5013	-83.329	4/18/2015
Peb2	37	15	42.5152	-83.344	4/26/2008
Peb2	24	9	42.5152	-83.344	4/21/2012
Peb3	37	16	42.5009	-83.325	5/2/2012
Peb3	28	11	42.5009	-83.325	4/18/2015

FIELDID	SQI	# Taxa	Latitude	Longitude	Collection Date
Ton1	46	16	42.36701	-83.499	4/13/2008
Ton1	57	22	42.36701	-83.499	5/24/2012
Ton1	44	16	42.36701	-83.499	4/18/2015
Ton1/2	44	18	42.36437	-83.4894	4/12/2012
Ton1/2	26	11	42.36437	-83.4894	4/18/2015
Up1	20	8	42.47526	-83.3857	4/29/2006
Up1	17	7	42.47526	-83.3857	4/26/2008
Up1	28	15	42.47526	-83.3857	4/21/2012
Up1	30	11	42.47526	-83.3857	4/18/2015
Up2	17	8	42.46323	-83.3681	4/21/2006
Up2	18	8	42.46323	-83.3681	4/26/2008
Up2	26	9	42.46323	-83.3681	4/21/2012
Wall0	36	14	42.43673	-83.4737	4/26/2008
Wall0	18	8	42.43673	-83.4737	4/21/2012
Wall0	21	9	42.43673	-83.4737	4/18/2015
Wall1	31	10	42.44957	-83.4653	4/26/2008
Wall1	30	12	42.44957	-83.4653	4/21/2012
Wall2	18	8	42.46732	-83.4662	4/26/2008
Wall3	31	12	42.49486	-83.4958	4/18/2015
Wall4	30	10	42.43354	-83.4806	4/26/2008

Appendix 4: ANOVA Tests for Trends in Spatial and Temporal Metal Accumulation

Aluminum by Branch

Anova: Single Factor

Groups	Count	Sum	Average	Variance
Lower Log(ppm+1)	22	62.58474	2.844761	0.057682
Middle Log(ppm+1)	73	190.3689	2.607793	0.095995
Main Log(ppm+1)	56	142.3118	2.541282	0.075627
Upper Log(ppm+1)	30	80.85896	2.695299	0.104231

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1.619329	3	0.539776	6.242374	0.000472	2.655647
Within Groups	15.30514	177	0.08647			
Total	16.92447	180				

Manganese by Branch

Anova: Single Factor

Groups	Count	Sum	Average	Variance
Lower Log(ppm+1)	23	71.82734	3.122928	0.060378
Middle Log(ppm+1)	73	222.2892	3.045058	0.093657
Main Log(ppm+1)	56	162.8151	2.907412	0.126385
Upper Log(ppm+1)	30	90.80177	3.026726	0.130198

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.980750663	3	0.326917	3.095511	0.028276	2.655359
Within Groups	18.7985755	178	0.10561			
Total	19.77932616	181				

Cobalt by Branch

Anova: Single Factor

Groups	Count	Sum	Average	Variance
Lower Log(ppm+1)	23	11.27863	0.490375	0.047015
Middle Log(ppm+1)	73	27.167	0.372151	0.026708
Main Log(ppm+1)	56	20.53523	0.3667	0.019137
Upper Log(ppm+1)	30	11.27152	0.375717	0.024423

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.288599	3	0.0962	3.629327	0.014114	2.655359
Within Groups	4.718103	178	0.026506			
Total	5.006702	181				

Arsenic by Branch

Anova: Single Factor

Groups	Count	Sum	Average	Variance
Lower Log(ppm+1)	23	10.45073	0.45438	0.028306
Middle Log(ppm+1)	73	32.02568	0.438708	0.027998
Main Log(ppm+1)	56	30.02101	0.536089	0.04808
Upper Log(ppm+1)	30	14.06742	0.468914	0.022408

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.315955	3	0.105318	3.159795	0.02601	2.655359
Within Groups	5.932876	178	0.033331			
Total	6.248831	181				

Nickel by Branch

Anova: Single Factor

Groups	Count	Sum	Average	Variance
Lower Log(ppm+1)	23	16.66497	0.724564	0.017888
Middle Log(ppm+1)	73	46.55032	0.637676	0.029708
Main Log(ppm+1)	56	34.86443	0.622579	0.014006
Upper Log(ppm+1)	30	20.32282	0.677427	0.019137

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.20358	3	0.06786	3.131076	0.026999	2.655359
Within Groups	3.857805	178	0.021673			
Total	4.061385	181				

Selenium by Branch

Anova: Single Factor

Groups	Count	Sum	Average	Variance
Lower Log(ppm+1)	23	8.799346	0.38258	0.006731
Middle Log(ppm+1)	73	28.05428	0.384305	0.018221
Main Log(ppm+1)	56	24.84964	0.443744	0.008024
Upper Log(ppm+1)	30	10.92851	0.364284	0.021995

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.170232	3	0.056744	3.977818	0.008959	2.655359
Within Groups	2.539187	178	0.014265			
Total	2.709419	181				

Sodium by Branch

Anova: Single Factor

Groups	Count	Sum	Average	Variance
Lower Log(ppm+1)	23	85.07812	3.699049	0.117428
Middle Log(ppm+1)	73	262.739	3.599164	0.096413
Main Log(ppm+1)	56	196.7646	3.513653	0.04498
Upper Log(ppm+1)	30	110.1583	3.671944	0.123631

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.797335	3	0.265778	3.035644	0.030562	2.655359
Within Groups	15.58436	178	0.087553			
Total	16.38169	181				

Magnesium by Branch

Anova: Single Factor

Groups	Count	Sum	Average	Variance
Lower Log(ppm+1)	23	72.80992	3.165649	0.023135
Middle Log(ppm+1)	73	225.1102	3.083702	0.029941
Main Log(ppm+1)	56	176.7455	3.156169	0.028519
Upper Log(ppm+1)	30	95.79623	3.193208	0.03415

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.340379	3	0.11346	3.866288	0.010362	2.655359
Within Groups	5.223568	178	0.029346			
Total	5.563947	181				

Potassium by Branch

Anova: Single Factor

Groups	Count	Sum	Average	Variance
Lower Log(ppm+1)	23	77.8966	3.386809	0.012231
Middle Log(ppm+1)	73	245.8886	3.368337	0.058662
Main Log(ppm+1)	56	184.4318	3.293424	0.056061
Upper Log(ppm+1)	30	104.0329	3.467763	0.017487

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.615274	3	0.205091	4.516294	0.004437	2.655359
Within Groups	8.083236	178	0.045411			
Total	8.69851	181				

Calcium by Branch

Anova: Single Factor

Groups	Count	Sum	Average	Variance
Lower Log(ppm+1)	23	84.94493	3.693258	0.071614
Middle Log(ppm+1)	73	272.8556	3.737747	0.043147
Main Log(ppm+1)	56	217.2452	3.879379	0.075689
Upper Log(ppm+1)	30	112.4216	3.747388	0.064494

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.887028	3	0.295676	4.911696	0.00265	2.655359
Within Groups	10.7153	178	0.060198			
Total	11.60233	181				

Iron by Branch

Anova: Single Factor

Groups	Count	Sum	Average	Variance
Lower Log(ppm+1)	23	57.23564	2.488506	0.066125
Middle Log(ppm+1)	73	169.6764	2.324335	0.057569
Main Log(ppm+1)	56	130.7552	2.334915	0.059047
Upper Log(ppm+1)	30	71.02882	2.367627	0.079883

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.50593	3	0.168643	2.688886	0.047882	2.655359
Within Groups	11.16393	178	0.062719			
Total	11.66986	181				

Aluminum by Branch

Anova: Single Factor

Groups	*Count*	*Sum*	*Average*	*Variance*
2006 Al27 Log(ppm+1)	35	89.8896	2.56827	0.09679
2008 Al27 Log(ppm+1)	42	110.593	2.63318	0.07928
2012 Al27 Log(ppm+1)	42	105.134	2.50320	0.06578
2012 Al27 Log(ppm+1)	61	167.826	2.75125	0.09967

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.70585107	3	0.56861	6.57650	0.00030	2.65593
Within Groups	15.2172877	176	0.08646			
Total	16.923138	179				

Manganese Rouge Watershed

Anova: Single Factor

Groups	*Count*	*Sum*	*Average*	*Variance*
2006 Mn Log(ppm+1)	35	98.64245	2.818356	0.139686699
2008 Mn Log(ppm+1)	42	124.7799	2.97095	0.085015519
2012 Mn Log(ppm+1)	44	128.0012	2.909119	0.052296759
2015 Mn Log(ppm+1)	61	196.3098	3.218194	0.080905159

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	4.441272	3	1.480424	17.18050172	7.6E-10	2.655359
Within Groups	15.33805	178	0.086169			
Total	19.77933	181				

Cobalt Rouge Watershed

Anova: Single Factor

Groups	*Count*	*Sum*	*Average*	*Variance*
2006 Co Log(ppm+1)	35	11.32085	0.323453	0.009801
2008 Co Log(ppm+1)	42	16.71608	0.398002	0.014671
2012 Co Log(ppm+1)	44	14.11475	0.32079	0.010432
2015 Co Log(ppm+1)	61	28.1007	0.460667	0.049221

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.670165	3	0.223388	9.169334	1.129E-05	2.655359
Within Groups	4.336536	178	0.024363			
Total	5.006702	181				

Arsenic Rouge Watershed

Anova: Single Factor

Groups	Count	Sum	Average	Variance
2006 As Log(ppm+1)	35	15.04973	0.429992	0.020485
2008 As Log(ppm+1)	42	23.40794	0.557332	0.045152
2012 As Log(ppm+1)	44	17.38821	0.395187	0.012991
2015 As Log(ppm+1)	61	30.71896	0.50359	0.040946

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.685673	3	0.228558	7.312982	0.000119	2.655359
Within Groups	5.563158	178	0.031254			
Total	6.248831	181				

Strontium Rouge Watershed

Anova: Single Factor

Groups	Count	Sum	Average	Variance
2006 Sr Log(ppm+1)	35	43.99244	1.256927	0.056569
2008 Sr Log(ppm+1)	42	58.27166	1.387421	0.044913
2012 Sr Log(ppm+1)	44	63.38791	1.440634	0.067534
2015 Sr Log(ppm+1)	61	87.84908	1.440149	0.045791

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.884803	3	0.294934	5.575308	0.001118	2.655359
Within Groups	9.416215	178	0.0529			
Total	10.30102	181				

Cadmium Rouge Watershed

Anova: Single Factor

Groups	Count	Sum	Average	Variance
2006 Cd Log(ppm+1)	35	3.585442	0.102441	0.005269
2008 Cd Log(ppm+1)	42	3.366458	0.080154	0.001159
2012 Cd Log(ppm+1)	44	2.569039	0.058387	0.001061
2015 Cd Log(ppm+1)	61	4.227475	0.069303	0.002288

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.041541	3	0.013847	6.017968	0.00063	2.655359
Within Groups	0.40957	178	0.002301			
Total	0.451111	181				

Barium Rouge Watershed

Anova: Single Factor

Groups	*Count*	*Sum*	*Average*	*Variance*
2006 Ba Log(ppm+1)	35	48.69968	1.391419	0.09378
2008 Ba Log(ppm+1)	42	69.43855	1.653299	0.092561
2012 Ba Log(ppm+1)	44	67.1424	1.525964	0.08584
2015 Ba Log(ppm+1)	61	103.3636	1.694486	0.061237

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2.391886773	3	0.797296	9.890576	4.5E-06	2.655359
Within Groups	14.34887334	178	0.080612			
Total	16.74076011	181				

Lead Rouge Watershed

Anova: Single Factor

Groups	*Count*	*Sum*	*Average*	*Variance*
2006 Pb Log(ppm+1)	35	22.21209	0.634631	0.039925
2008 Pb Log(ppm+1)	42	31.31779	0.745662	0.074288
2012 Pb Log(ppm+1)	44	24.62077	0.559563	0.073818
2012 Pb Log(ppm+1)	61	36.69481	0.601554	0.038316

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.83008	3	0.276693	4.986788	0.002403	2.655359
Within Groups	9.876382	178	0.055485			
Total	10.70646	181				

Sodium Rouge Watershed

Anova: Single Factor

Groups	*Count*	*Sum*	*Average*	*Variance*
2006 Na Log(ppm+1)	35	124.1517	3.547191	0.119713
2008 Na Log(ppm+1)	42	148.9207	3.545732	0.048034
2012 Na Log(ppm+1)	44	150.9437	3.430539	0.02936
2015 Na Log(ppm+1)	61	230.7238	3.782358	0.09279

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3.512212	3	1.170737	16.19267	2.3E-09	2.655359
Within Groups	12.86948	178	0.0723			
Total	16.38169	181				

Potassium Rouge Watershed

Anova: Single Factor

Groups	Count	Sum	Average	Variance
2006 K Log(ppm+1)	35	116.371	3.324885	0.068216
2008 K Log(ppm+1)	42	140.5098	3.345472	0.07692
2012 K Log(ppm+1)	44	146.2656	3.324219	0.023349
2015 K Log(ppm+1)	61	209.1034	3.427925	0.030577

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.386873	3	0.128958	2.761726	0.043581	2.655359
Within Groups	8.311637	178	0.046695			
Total	8.69851	181				

Iron Rouge Watershed

Anova: Single Factor

Groups	Count	Sum	Average	Variance
2006 Fe Log(ppm+1)	35	114.3175	3.266215	0.061869
2008 Fe Log(ppm+1)	42	144.1215	3.431464	0.063751
2012 Fe Log(ppm+1)	44	141.8731	3.224389	0.03917
2015 Fe Log(ppm+1)	61	210.0129	3.442835	0.060513

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1.742235	3	0.580745	10.30381	2.7E-06	2.655359
Within Groups	10.03246	178	0.056362			
Total	11.7747	181				

Chromium Main Branch

Anova: Single Factor

Groups	Count	Sum	Average	Variance
2006 Log(ppm+1)	10	5.876728	0.587673	0.043905
2008 Log(ppm+1)	14	10.88379	0.777413	0.016695
2012 Log(ppm+1)	20	14.29162	0.714581	0.012584
2015 Log(ppm+1)	12	7.561709	0.630142	0.016903

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.266751	3	0.088917	4.457801	0.00734	2.7826
Within Groups	1.037214	52	0.019946			
Total	1.303965	55				

Manganese Main Branch

Anova: Single Factor

Groups	*Count*	*Sum*	*Average*	*Variance*
2006 Log(ppm+1)	10	25.20117	2.520117	0.110947
2008 Log(ppm+1)	14	41.96042	2.997173	0.093118
2012 Log(ppm+1)	20	57.55435	2.877718	0.08498
2015 Log(ppm+1)	12	38.09912	3.174927	0.058029

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2.489179	3	0.829726	9.669599	3.55E-05	2.7826
Within Groups	4.462002	52	0.085808			
Total	6.951181	55				

Nickel Main Branch

Anova: Single Factor

Groups	*Count*	*Sum*	*Average*	*Variance*
2006 Log(ppm+1)	10	6.692478	0.669248	0.011457
2008 Log(ppm+1)	14	9.87516	0.705369	0.019654
2012 Log(ppm+1)	20	11.67386	0.583693	0.008575
2015 Log(ppm+1)	12	6.622934	0.551911	0.003718

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.207907	3	0.069302	6.407397	0.000892	2.7826
Within Groups	0.562432	52	0.010816			
Total	0.770339	55				

Copper Main Branch

Anova: Single Factor

Groups	*Count*	*Sum*	*Average*	*Variance*
2006 Log(ppm+1)	10	15.4731	1.54731	0.007865
2008 Log(ppm+1)	14	21.99474	1.571053	0.013494
2012 Log(ppm+1)	20	29.98464	1.499232	0.012252
2015 Log(ppm+1)	12	17.47552	1.456293	0.010155

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.100618	3	0.033539	2.952518	0.041003	2.7826
Within Groups	0.590699	52	0.01136			
Total	0.691318	55				

Arsenic Main Branch

Anova: Single Factor

Groups	*Count*	*Sum*	*Average*	*Variance*
2006 Log(ppm+1)	10	4.867961	0.486796	0.01816
2008 Log(ppm+1)	14	10.11383	0.722416	0.065229
2012 Log(ppm+1)	20	8.759702	0.437985	0.015209
2015 Log(ppm+1)	12	6.279516	0.523293	0.058111

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.704801	3	0.234934	6.298438	0.000999	2.7826
Within Groups	1.939617	52	0.0373			
Total	2.644418	55				

Cadmium Main Branch

Anova: Single Factor

Groups	*Count*	*Sum*	*Average*	*Variance*
2006 Log(ppm+1)	10	1.156021	0.115602	0.008273
2008 Log(ppm+1)	14	0.980765	0.070055	0.000479
2012 Log(ppm+1)	20	1.222871	0.061144	0.001507
2015 Log(ppm+1)	12	0.764537	0.063711	0.000625

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.022094	3	0.007365	3.295687	0.027529	2.7826
Within Groups	0.116203	52	0.002235			
Total	0.138297	55				

Barium Main Branch

Anova: Single Factor

Groups	*Count*	*Sum*	*Average*	*Variance*
2006 Log(ppm+1)	10	12.48594	1.248594	0.207569
2008 Log(ppm+1)	14	23.4276	1.6734	0.047506
2012 Log(ppm+1)	20	30.30284	1.515142	0.104126
2015 Log(ppm+1)	12	19.43242	1.619369	0.021716

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.180022	3	0.393341	4.349108	0.008289	2.7826
Within Groups	4.702966	52	0.090442			
Total	5.882988	55				

Lead Main Branch

Anova: Single Factor				
Groups	*Count*	*Sum*	*Average*	*Variance*
2006 Log(ppm+1)	10	6.225117	0.622512	0.025713
2008 Log(ppm+1)	14	10.52306	0.751647	0.013195
2012 Log(ppm+1)	20	10.52896	0.526448	0.026542
2015 Log(ppm+1)	12	6.557412	0.546451	0.006798

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.468649	3	0.156216	8.271869	0.000135	2.7826
Within Groups	0.982033	52	0.018885			
Total	1.450681	55				

Sodium Main Branch

Anova: Single Factor				
Groups	*Count*	*Sum*	*Average*	*Variance*
2006 Log(ppm+1)	10	33.99099	3.399099	0.043949
2008 Log(ppm+1)	14	50.73342	3.623815	0.04478
2012 Log(ppm+1)	20	68.26067	3.413034	0.019834
2015 Log(ppm+1)	12	43.7795	3.648292	0.036202

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.721143	3	0.240381	7.131588	0.000422	2.7826
Within Groups	1.752739	52	0.033707			
Total	2.473882	55				

Potassium Main Branch

Anova: Single Factor				
Groups	*Count*	*Sum*	*Average*	*Variance*
2006 Log(ppm+1)	10	31.14768	3.114768	0.143477
2008 Log(ppm+1)	14	46.88435	3.348882	0.056007
2012 Log(ppm+1)	20	65.75918	3.287959	0.023274
2015 Log(ppm+1)	12	40.64056	3.386714	0.014044

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.467272	3	0.155757	3.095985	0.034699	2.7826
Within Groups	2.616093	52	0.050309			
Total	3.083366	55				

Iron Main Branch

Anova: Single Factor

Groups	*Count*	*Sum*	*Average*	*Variance*
2006 Log(ppm+1)	10	32.46297	3.246297	0.061802
2008 Log(ppm+1)	14	49.23838	3.517027	0.039658
2012 Log(ppm+1)	20	64.84277	3.242139	0.057529
2015 Log(ppm+1)	12	40.09258	3.341048	0.036301

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.715186	3	0.238395	4.8346	0.00483	2.7826
Within Groups	2.564134	52	0.04931			
Total	3.27932	55				

Aluminum Upper Branch

Anova: Single Factor

Groups	*Count*	*Sum*	*Average*	*Variance*
2006 Log(ppm+1)	8	20.22156	2.527695	0.072
2008 Log(ppm+1)	6	16.84857	2.808095	0.1352
2012 Log(ppm+1)	6	14.62972	2.438286	0.0715
2015 Log(ppm+1)	10	29.15910	2.915910	0.0327

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.184088	3	0.3946961	5.58145	0.00430	2.9751539
Within Groups	1.8386056	26	0.070715			
Total	3.0226941	29				

Arsenic Upper Branch

Anova: Single Factor

Groups	*Count*	*Sum*	*Average*	*Variance*
2006 Log(ppm+1)	8	3.835268	0.479408	0.030752
2008 Log(ppm+1)	6	2.934463	0.489077	0.013616
2012 Log(ppm+1)	6	1.820136	0.303356	0.007403
2015 Log(ppm+1)	10	5.477549	0.547755	0.011059

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.229935883	3	0.076645	4.745869	0.009047	2.975154
Within Groups	0.419897354	26	0.01615			
Total	0.649833237	29				

Cobalt Upper Branch

Anova: Single Factor

Groups	Count	Sum	Average	Variance
2006 Log(ppm+1)	8	2.418173	0.302272	0.009045
2008 Log(ppm+1)	6	2.465656	0.410943	0.026844
2012 Log(ppm+1)	6	1.433684	0.238947	0.004772
2015 Log(ppm+1)	10	4.954004	0.4954	0.020087

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.306076	3	0.102025	6.595674	0.001835	2.975154
Within Groups	0.402181	26	0.015469			
Total	0.708257	29				

Barium Upper Branch

Anova: Single Factor

Groups	Count	Sum	Average	Variance
2006 Log(ppm+1)	8	11.11498	1.389372	0.070787
2008 Log(ppm+1)	6	8.913898	1.48565	0.058838
2012 Log(ppm+1)	6	9.290172	1.548362	0.060338
2015 Log(ppm+1)	10	17.73819	1.773819	0.038294

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.721871	3	0.240624	4.35659	0.012964	2.975154
Within Groups	1.436035	26	0.055232			
Total	2.157907	29				

Iron Upper Branch

Anova: Single Factor

Groups	Count	Sum	Average	Variance
2006 Log(ppm+1)	8	25.72169	3.215212	0.043826
2008 Log(ppm+1)	6	20.86731	3.477884	0.188405
2012 Log(ppm+1)	6	19.00474	3.167457	0.03659
2015 Log(ppm+1)	10	35.37274	3.537274	0.013441

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.786864	3	0.262288	4.391946	0.012543	2.975154
Within Groups	1.552726	26	0.05972			
Total	2.33959	29				

Manganese Middle Branch

Anova: Single Factor

Groups	Count	Sum	Average	Variance
2006 Log(ppm+1)	11	31.93982	2.90362	0.091414
2008 Log(ppm+1)	20	58.90835	2.945417	0.076837
2012 Log(ppm+1)	16	47.05638	2.941024	0.022668
2015 Log(ppm+1)	26	83.38509	3.207119	0.07335

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1.260955	3	0.420318	6.377125	0.000705	2.737492
Within Groups	4.547811	69	0.06591			
Total	5.808765	72				

Arsenic Middle Branch

Anova: Single Factor

Groups	Count	Sum	Average	Variance
2006 Log(ppm+1)	11	3.766246	0.342386	0.008495
2008 Log(ppm+1)	20	9.710496	0.485525	0.016537
2012 Log(ppm+1)	16	5.993713	0.374607	0.009151
2015 Log(ppm+1)	26	11.73106	0.451194	0.023831

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.206385	3	0.068795	4.192596	0.008739	2.737492
Within Groups	1.1322	69	0.016409			
Total	1.338585	72				

Selenium Middle Branch

Anova: Single Factor

Groups	Count	Sum	Average	Variance
2006 Log(ppm+1)	11	4.086205	0.371473	0.001054
2008 Log(ppm+1)	20	8.03492	0.401746	0.003322
2012 Log(ppm+1)	16	7.076475	0.44228	0.035587
2015 Log(ppm+1)	26	8.75016	0.336545	0.006412

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.120824	3	0.040275	3.619611	0.017283	2.737492
Within Groups	0.767749	69	0.011127			
Total	0.888573	72				

Strontium Middle Branch

Anova: Single Factor

Groups	Count	Sum	Average	Variance
2006 Log(ppm+1)	11	13.60146	1.236497	0.027839
2008 Log(ppm+1)	20	26.42268	1.321134	0.034609
2012 Log(ppm+1)	16	23.01091	1.438182	0.049722
2015 Log(ppm+1)	26	37.07477	1.425953	0.02854

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.399454	3	0.133151	3.83564	0.013353	2.737492
Within Groups	2.395283	69	0.034714			
Total	2.794737	72				

Cadmium Middle Branch

Anova: Single Factor

Groups	Count	Sum	Average	Variance
2006 Log(ppm+1)	11	1.231861	0.111987	0.00736
2008 Log(ppm+1)	20	1.760611	0.088031	0.001656
2012 Log(ppm+1)	16	0.877593	0.05485	0.000575
2015 Log(ppm+1)	26	1.642305	0.063166	0.001539

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.028564	3	0.009521	4.317728	0.007538	2.737492
Within Groups	0.152154	69	0.002205			
Total	0.180718	72				

Barium Middle Branch

Anova: Single Factor

Groups	Count	Sum	Average	Variance
2006 Log(ppm+1)	11	16.10968	1.464516	0.04284
2008 Log(ppm+1)	20	32.07086	1.603543	0.06214
2012 Log(ppm+1)	16	23.73575	1.483484	0.048198
2015 Log(ppm+1)	26	44.22364	1.700909	0.059771

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.678161	3	0.226054	4.076455	0.010028	2.737492
Within Groups	3.826294	69	0.055454			
Total	4.504455	72				

Sodium Middle Branch

Anova: Single Factor

Groups	Count	Sum	Average	Variance
2006 Log(ppm+1)	11	38.6099	3.509991	0.04132
2008 Log(ppm+1)	20	69.66471	3.483235	0.049826
2012 Log(ppm+1)	16	55.35696	3.45981	0.051554
2015 Log(ppm+1)	26	98.10743	3.773363	0.06154

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1.442246	3	0.480749	9.034388	4E-05	2.737492
Within Groups	3.671712	69	0.053213			
Total	5.113958	72				

Calcium Middle Branch

Anova: Single Factor

Groups	Count	Sum	Average	Variance
2006 Log(ppm+1)	11	40.30952	3.664502	0.022674
2008 Log(ppm+1)	20	72.87199	3.643599	0.031565
2012 Log(ppm+1)	16	60.0864	3.7554	0.036909
2015 Log(ppm+1)	26	98.58774	3.791836	0.027435

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.303646	3	0.101215	3.380426	0.023023	2.737492
Within Groups	2.065969	69	0.029942			
Total	2.369615	72				

Iron Middle Branch

Anova: Single Factor

Groups	Count	Sum	Average	Variance
2006 Log(ppm+1)	11	35.13372	3.193975	0.047149
2008 Log(ppm+1)	20	67.37866	3.368933	0.047697
2012 Log(ppm+1)	16	51.40055	3.212534	0.024301
2015 Log(ppm+1)	26	87.60771	3.369527	0.044241

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.461503	3	0.153834	3.726675	0.015206	2.737492
Within Groups	2.848268	69	0.041279			
Total	3.309771	72				

Strontium Lower Branch

Anova: Single Factor

Groups	Count	Sum	Average	Variance
2006 Log(ppm+1)	6	7.320727	1.220121	0.041945
2008 Log(ppm+1)	2	3.514518	1.757259	0.059322
2012 Log(ppm+1)	2	3.036597	1.518298	0.010952
2015 Log(ppm+1)	13	18.28229	1.40633	0.055542

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.477824	3	0.159275	3.197256	0.046889	3.12735
Within Groups	0.946506	19	0.049816			
Total	1.42433	22				

Barium Lower Branch

Anova: Single Factor

Groups	Count	Sum	Average	Variance
2006 Log(ppm+1)	6	8.989083	1.498181	0.013061
2008 Log(ppm+1)	2	5.026189	2.513095	0.000272
2012 Log(ppm+1)	2	3.813642	1.906821	0.363743
2015 Log(ppm+1)	13	20.98255	1.614042	0.069419

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1.744616	3	0.581539	8.752948	0.000746	3.12735
Within Groups	1.262344	19	0.066439			
Total	3.00696	22				

Sodium Lower Branch

Anova: Single Factor

SUMMARY

Groups	Count	Sum	Average	Variance
2006 Log(ppm+1)	6	20.62581	3.437635	0.041345
2008 Log(ppm+1)	2	7.08002	3.54001	0.120256
2012 Log(ppm+1)	2	6.848984	3.424492	0.003959
2015 Log(ppm+1)	13	50.52331	3.886408	0.09873

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1.067721	3	0.355907	4.461455	0.015593	3.12735
Within Groups	1.515702	19	0.079774			
Total	2.583423	22				

Potassium Lower Branch

Anova: Single Factor

Groups	Count	Sum	Average	Variance
2006 Log(ppm+1)	6	20.03888	3.339813	0.004029
2008 Log(ppm+1)	2	6.32113	3.160565	0.002885
2012 Log(ppm+1)	2	6.80791	3.403955	0.001116
2015 Log(ppm+1)	13	44.72868	3.440668	0.007584

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.153922	3	0.051307	8.465235	0.000891	3.12735
Within Groups	0.115158	19	0.006061			
Total	0.26908	22				

Appendix 5: T-Tests for Protruded Anal Papillae

t-Test: Two-Sample Assuming Unequal Variances		
Aluminum	*Protruded Log(ppm+1)*	*Normal Log(ppm+1)*
Mean	1.591167355	1.645380042
Variance	0.07331224	0.090335168
Observations	10	172
Hypothesized Mean Difference	0	
df	10	
t Stat	-0.611629397	
P(T<=t) two-tail	0.554432486	
t Critical two-tail	2.228138852	

t-Test: Two-Sample Assuming Unequal Variances		
Chromium	*Protruded Log(ppm+1)*	*Normal Log(ppm+1)*
Mean	0.133842282	0.160928965
Variance	0.001705416	0.006235789
Observations	10	172
Hypothesized Mean Difference	0	
df	13	
t Stat	-1.883582138	
P(T<=t) two-tail	0.082183674	
t Critical two-tail	2.160368656	

t-Test: Two-Sample Assuming Unequal Variances		
Manganese	*Protruded Log(ppm+1)*	*Normal Log(ppm+1)*
Mean	1.914140293	2.020511031
Variance	0.112357654	0.106132987
Observations	10	172
Hypothesized Mean Difference	0	
df	10	
t Stat	-0.97703781	
P(T<=t) two-tail	0.351593984	
t Critical two-tail	2.228138852	

t-Test: Two-Sample Assuming Unequal Variances		
Cobalt	*Protruded Log(ppm+1)*	*Normal Log(ppm+1)*
Mean	0.080025909	0.063079224
Variance	0.005860866	0.00213846
Observations	10	172
Hypothesized Mean Difference	0	
df	9	
t Stat	0.692700643	
P(T<=t) two-tail	0.505976494	
t Critical two-tail	2.262157163	

t-Test: Two-Sample Assuming Unequal Variances		
Copper	*Protruded Log(ppm+1)*	*Normal Log(ppm+1)*
Mean	0.59665159	0.607863461
Variance	0.010575579	0.018552117
Observations	10	172
Hypothesized Mean Difference	0	
df	11	
t Stat	-0.328425622	
P(T<=t) two-tail	0.748756983	
t Critical two-tail	2.20098516	

t-Test: Two-Sample Assuming Unequal Variances		
Nickel	*Protruded Log(ppm+1)*	*Normal Log(ppm+1)*
Mean	0.136488071	0.135175497
Variance	0.001910741	0.003701336
Observations	10	172
Hypothesized Mean Difference	0	
df	11	
t Stat	0.090022049	
P(T<=t) two-tail	0.929887945	
t Critical two-tail	2.20098516	

t-Test: Two-Sample Assuming Unequal Variances		
Zinc	*Protruded Log(ppm+1)*	*Normal Log(ppm+1)*
Mean	1.188658206	1.185835818
Variance	0.008783258	0.025968641
Observations	10	172
Hypothesized Mean Difference	0	
df	12	
t Stat	0.087971996	
P(T<=t) two-tail	0.931349976	
t Critical two-tail	2.17881283	

t-Test: Two-Sample Assuming Unequal Variances		
Arsenic	*Protruded Log(ppm+1)*	*Normal Log(ppm+1)*
Mean	0.075308912	0.087467053
Variance	0.001886509	0.004132
Observations	10	172
Hypothesized Mean Difference	0	
df	11	
t Stat	-0.833700074	
P(T<=t) two-tail	0.422184644	
t Critical two-tail	2.20098516	

t-Test: Two-Sample Assuming Unequal Variances		
Selenium	*Protruded Log(ppm+1)*	*Normal Log(ppm+1)*
Mean	0.057322836	0.064498706
Variance	0.000233167	0.00148216
Observations	10	172
Hypothesized Mean Difference	0	
df	17	
t Stat	-1.269839231	
P(T<=t) two-tail	0.221249248	
t Critical two-tail	2.109815578	

t-Test: Two-Sample Assuming Unequal Variances		
Cadmium	*Protruded Log(ppm+1)*	*Normal Log(ppm+1)*
Mean	0.009016445	0.008459078
Variance	3.09965E-05	4.56359E-05
Observations	10	172
Hypothesized Mean Difference	0	
df	11	
t Stat	0.303843816	
P(T<=t) two-tail	0.766917103	
t Critical two-tail	2.20098516	

t-Test: Two-Sample Assuming Unequal Variances		
Cadmium	***Protruded Log(ppm+1)***	***Normal Log(ppm+1)***
Mean	**0.009016445**	**0.008459078**
Variance	**3.09965E-05**	**4.56359E-05**
Observations	**10**	**172**
Hypothesized Mean Difference	**0**	
df	**11**	
t Stat	**0.303843816**	
P(T<=t) two-tail	**0.766917103**	
t Critical two-tail	**2.20098516**	

t-Test: Two-Sample Assuming Unequal Variances		
Barium	*Protruded Log(ppm+1)*	*Normal Log(ppm+1)*
Mean	0.588903475	0.702133898
Variance	0.031499444	0.055951657
Observations	10	172
Hypothesized Mean Difference	0	
df	11	
t Stat	-1.920748205	
P(T<=t) two-tail	0.081049798	
t Critical two-tail	2.20098516	

t-Test: Two-Sample Assuming Unequal Variances		
Lead	*Protruded Log(ppm+1)*	*Normal Log(ppm+1)*
Mean	0.110191248	0.139651726
Variance	0.001348671	0.015715693
Observations	10	172
Hypothesized Mean Difference	0	
df	25	
t Stat	-1.958653335	
P(T<=t) two-tail	0.061398656	
t Critical two-tail	2.059538553	

t-Test: Two-Sample Assuming Unequal Variances		
Magnesium	*Protruded Log(ppm+1)*	*Normal Log(ppm+1)*
Mean	2.128769081	2.137983983
Variance	0.044017638	0.029824583
Observations	10	172
Hypothesized Mean Difference	0	
df	10	
t Stat	-0.13623461	
P(T<=t) two-tail	0.894339389	
t Critical two-tail	2.228138852	

t-Test: Two-Sample Assuming Unequal Variances		
Sodium	*Protruded Log(ppm+1)*	*Normal Log(ppm+1)*
Mean	2.594562601	2.598901812
Variance	0.054151397	0.092567869
Observations	10	172
Hypothesized Mean Difference	0	
df	11	
t Stat	-0.056238149	
P(T<=t) two-tail	0.956160605	
t Critical two-tail	2.20098516	

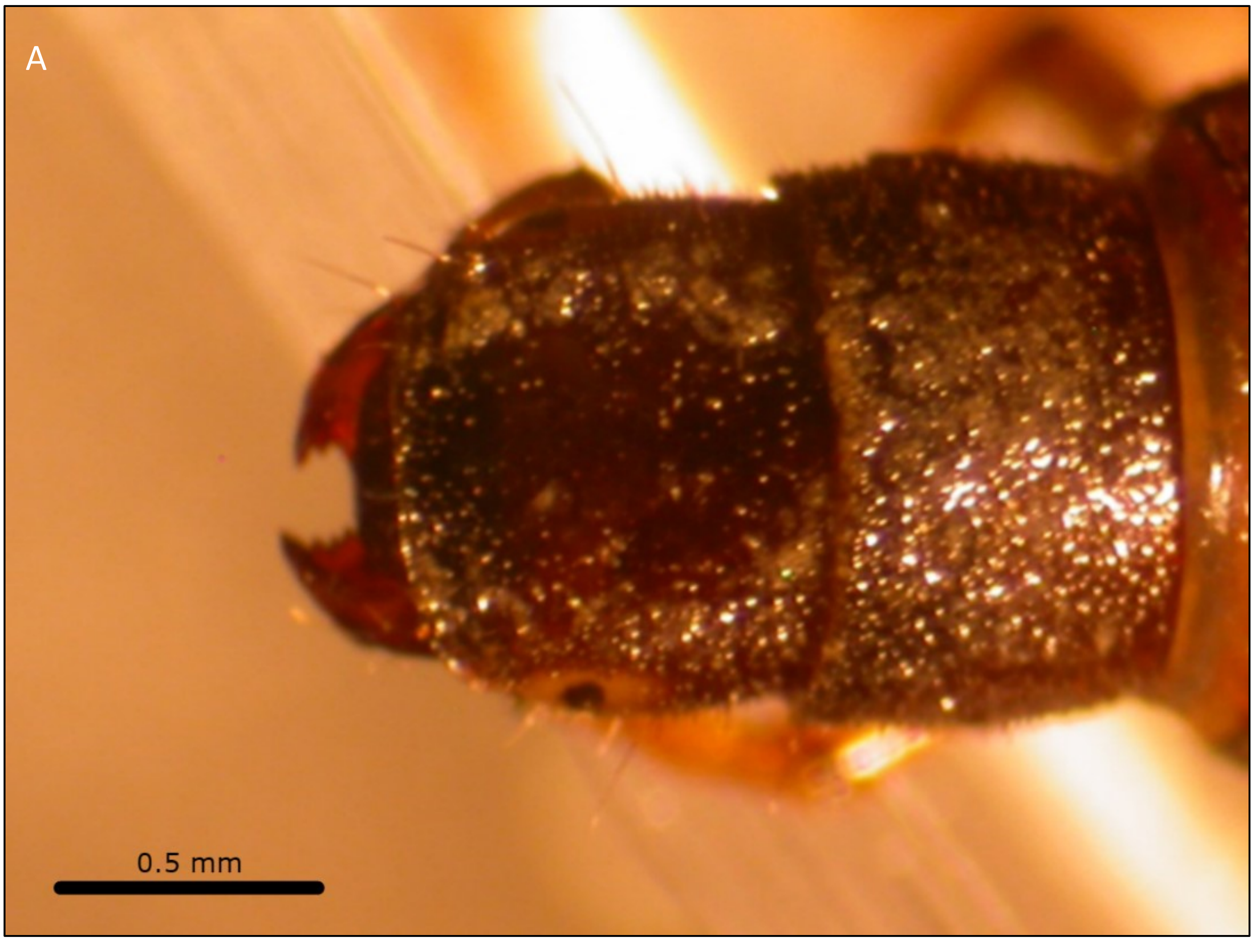
t-Test: Two-Sample Assuming Unequal Variances		
Potassium	*Protruded Log(ppm+1)*	*Normal Log(ppm+1)*
Mean	2.399095421	2.36410186
Variance	0.010216341	0.049451166
Observations	10	172
Hypothesized Mean Difference	0	
df	15	
t Stat	0.967152472	
P(T<=t) two-tail	0.348803888	
t Critical two-tail	2.131449546	

t-Test: Two-Sample Assuming Unequal Variances		
Potassium	*Protruded Log(ppm+1)*	*Normal Log(ppm+1)*
Mean	2.399095421	2.36410186
Variance	0.010216341	0.049451166
Observations	10	172
Hypothesized Mean Difference	0	
df	15	
t Stat	0.967152472	
P(T<=t) two-tail	0.348803888	
t Critical two-tail	2.131449546	

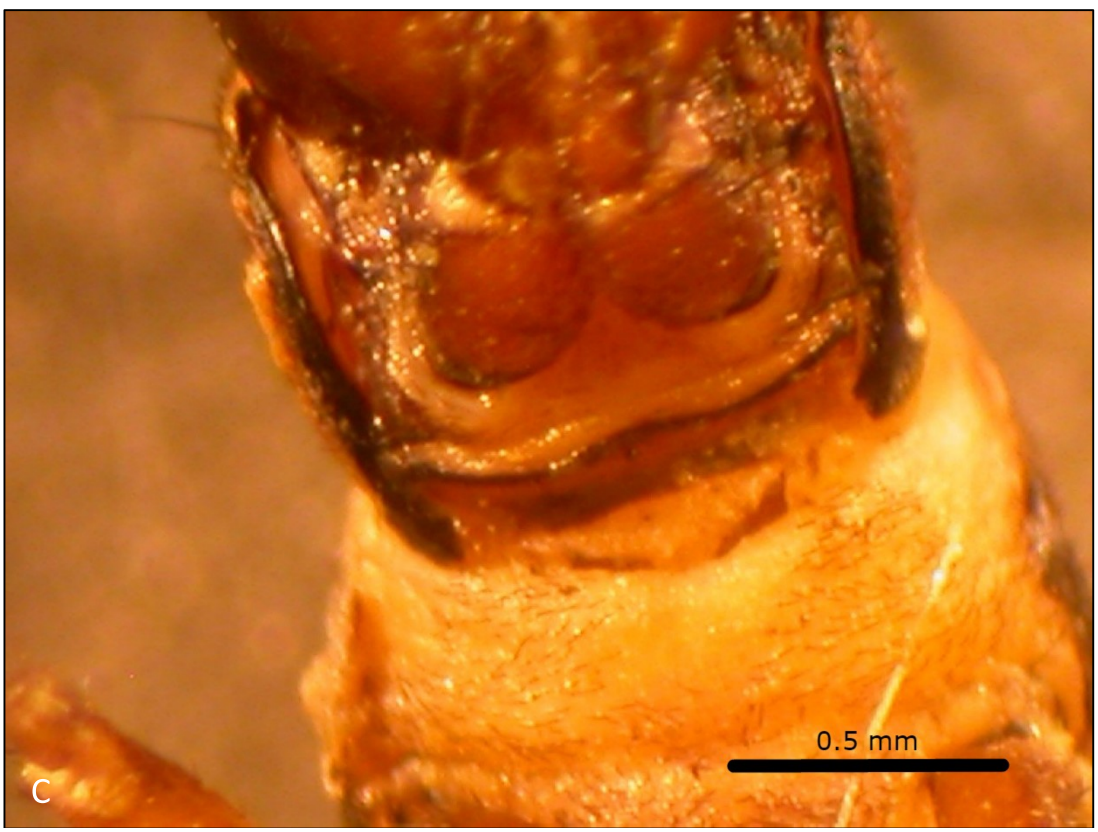
t-Test: Two-Sample Assuming Unequal Variances		
Iron	*Protruded Log(ppm+1)*	*Normal Log(ppm+1)*
Mean	2.286383252	2.359490119
Variance	0.10830703	0.06224903
Observations	10	172
Hypothesized Mean Difference	0	
df	10	
t Stat	-0.691022785	
P(T<=t) two-tail	0.505275267	
t Critical two-tail	2.228138852	

Appendix 6: Hydropsychidae Photographs

A light microscope was used to photograph the distinguishing features of the Hydropsychidae. The four photographs for the specimen show the following regions: lateral larval body (a), dorsal head (b), ventral thorax (c) and anal region (d). The ventral thorax is pictured between the first (most superior) and second pair of legs. Each specimen was classified at the genus level.




B


0.5 mm
C


D
0

Below each Hydropsychidae specimen identified with a unique ID #. The individuals of the Hydropsychidae family are labelled with their specific genus.

ID #	Genus	ID #	Genus	ID #	Genus
1	*Hydropsyche*	35	*Cheumatopsyche*	68	*Hydropsyche*
2	*Ceratopsyche*	36	*Hydropsyche*	69	*Hydropsyche*
3	*Ceratopsyche*	37	*Hydropsyche*	70	*Hydropsyche*
4	*Hydropsyche*	38	*Cheumatopsyche*	71	*Cheumatopsyche*
5	*Ceratopsyche*	39	*Cheumatopsyche*	72	*Cheumatopsyche*
6	*Ceratopsyche*	40	*Hydropsyche*	73	*Hydropsyche*
7	*Cheumatopsyche*	41	*Ceratopsyche*	74	*Hydropsyche*
8	*Cheumatopsyche*	42	*Ceratopsyche*	75	*Hydropsyche*
9	*Hydropsyche*	43	*Hydropsyche*	76	*Hydropsyche*
10	*Cheumatopsyche*	44	*Cheumatopsyche*	77	*Hydropsyche*
11	*Hydropsyche*	45	*Hydropsyche*	78	*Hydropsyche*
13	*Hydropsyche*	46	*Hydropsyche*	79	*Ceratopsyche*
14	*Hydropsyche*	47	*Hydropsyche*	80	*Ceratopsyche*
15	*Ceratopsyche*	48	*Ceratopsyche*	81	*Hydropsyche*
16	*Ceratopsyche*	49	*Cheumatopsyche*	82	*Hydropsyche*
17	*Ceratopsyche*	50	*Hydropsyche*	83	*Hydropsyche*
18	*Hydropsyche*	51	*Cheumatopsyche*	84	*Hydropsyche*
19	*Hydropsyche*	52	*Hydropsyche*	85	*Hydropsyche*
20	*Hydropsyche*	53	*Cheumatopsyche*	86	*Hydropsyche*
21	*Ceratopsyche*	54	*Ceratopsyche*	87	*Hydropsyche*
22	*Ceratopsyche*	55	*Hydropsyche*	88	*Hydropsyche*
23	*Hydropsyche*	56	*Hydropsyche*	89	*Hydropsyche*
24	*Cheumatopsyche*	57	*Cheumatopsyche*	90	*Hydropsyche*
25	*Hydropsyche*	58	*Cheumatopsyche*	91	*Hydropsyche*
26	*Hydropsyche*	59	*Hydropsyche*	92	*Ceratopsyche*
27	*Ceratopsyche*	60	*Cheumatopsyche*	93	*Hydropsyche*
28	*Ceratopsyche*	61	*Ceratopsyche*	94	*Hydropsyche*
29	*Hydropsyche*	62	*Cheumatopsyche*	95	*Hydropsyche*
30	*Hydropsyche*	63	*Cheumatopsyche*	96	*Hydropsyche*
31	*Hydropsyche*	64	*Hydropsyche*	97	*Hydropsyche*
32	*Hydropsyche*	65	*Hydropsyche*	98	*Ceratopsyche*
33	*Cheumatopsyche*	66	*Hydropsyche*	99	*Hydropsyche*
34	*Ceratopsyche*	67	*Hydropsyche*	100	*Hydropsyche*

ID #	Genus	ID #	Genus	ID #	Genus
101	*Hydropsyche*	135	*Hydropsyche*	171	*Cheumatopsyche*
102	*Hydropsyche*	136	*Hydropsyche*	172	*Hydropsyche*
103	*Hydropsyche*	137	*Hydropsyche*	173	*Cheumatopsyche*
104	*Hydropsyche*	138	*Ceratopsyche*	175	*Hydropsyche*
105	*Hydropsyche*	139	*Hydropsyche*	176	*Hydropsyche*
106	*Ceratopsyche*	140	*Ceratopsyche*	177	*Hydropsyche*
107	*Hydropsyche*	141	*Hydropsyche*	178	*Hydropsyche*
108	*Hydropsyche*	142	*Hydropsyche*	179	*Cheumatopsyche*
109	*Hydropsyche*	143	*Hydropsyche*	180	*Hydropsyche*
110	*Hydropsyche*	144	*Cheumatopsyche*	181	*Hydropsyche*
111	*Hydropsyche*	145	*Hydropsyche*	182	*Hydropsyche*
112	*Ceratopsyche*	146	*Hydropsyche*	183	*Cheumatopsyche*
113	*Hydropsyche*	147	*Hydropsyche*	184	*Cheumatopsyche*
114	*Hydropsyche*	148	*Hydropsyche*	185	*Hydropsyche*
115	*Cheumatopsyche*	149	*Hydropsyche*	186	*Ceratopsyche*
116	*Hydropsyche*	150	*Hydropsyche*		
117	*Hydropsyche*	151	*Hydropsyche*		
118	*Hydropsyche*	152	*Hydropsyche*		
119	*Hydropsyche*	153	*Cheumatopsyche*		
120	*Cheumatopsyche*	154	*Hydropsyche*		
121	*Hydropsyche*	155	*Hydropsyche*		
122	*Cheumatopsyche*	156	*Hydropsyche*		
123	*Hydropsyche*	157	*Hydropsyche*		
124	*Hydropsyche*	158	*Hydropsyche*		
125	*Hydropsyche*	159	*Hydropsyche*		
126	*Hydropsyche*	160	*Ceratopsyche*		
127	*Hydropsyche*	161	*Hydropsyche*		
128	*Hydropsyche*	162	*Cheumatopsyche*		
129	*Ceratopsyche*	163	*Hydropsyche*		
130	*Ceratopsyche*	164	*Cheumatopsyche*		
131	*Hydropsyche*	165	*Hydropsyche*		
132	*Hydropsyche*	166	*Hydropsyche*		
133	*Hydropsyche*	169	*Hydropsyche*		
134	*Hydropsyche*	170	*Hydropsyche*		